54 (1118)

The effect of moderately high atmospheric temperatures upon the formation of hemolysins.

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The experiments which have been reported in regard to the effect of high atmospheric temperatures upon susceptibility to bacterial infections, or upon the immunity reactions in response thereto, seem at first sight to be conflicting and unsatisfactory. Some authors report increased resistance as a result of external heat and others precisely the reverse. A more careful analysis shows however that if the several factors at work in such experiments and the various conditions employed by different investigators be considered, the results are reasonably harmonious. A moderate amount of heat may naturally be expected to produce a different result from temperatures so severe as to lead to a condition of fever in the experimental animals; and exposure to a hot atmosphere may produce one effect on the susceptibility of an animal to subsequent infection and quite another on the course of an infection already established.

The majority of investigators have been chiefly interested in the effect of the condition of fever upon recovery from infection, and have therefore exposed their animals to atmospheric conditions sufficiently extreme materially to increase the body temperature. Experiments of this kind have quite uniformly indicated that the progress of an infection already established is in greater or less degree checked by an artificial fever due to a very high atmospheric temperature, or produced by the Sachs-Aronson operation. Such experiments have been made and such a conclusion reached by Rovighi, Walther, Filehne, Hildebrandt, Loewy and Richter, Kast, Engelhardt, and Rolly and Meltzer. In all these experiments the high atmospheric temperatures used were 35°–41° C. and the body temperatures of the animals 40°–42°. Vincent and

Sacquépée and Loiseleur on the other hand found resistance lowered by high heating, but for the most part their experiments were concerned with the lighting up of latent infection or the invasion of bacteria from the digestive tract, a very different phenomenon from the progress of the struggle for immunity against an infection already established.

Finally there is another type of experiments in which the effects upon vital resistance of a moderately high temperature (30°–35°) have been studied; and these experiments yield results quite different from those which have just been reviewed. While a temperature approaching 40° by producing a state of fever appears to favor recovery from an infectious disease, a somewhat lower temperature seems to exert a lowering effect on general vital resistance without the compensating stimulation of vital processes which may accompany the development of fever. Five different investigations, the only ones with which we are familiar bearing on this point, all warrant the same conclusion. Fermi and Salsano (1892) found that a strain of avian tubercle bacilli which was incapable of producing a general infection in normal guinea pigs could be found in abundance in the glands of animals kept at 33°–35°. Similarly mice when heated showed many more tubercle bacilli, of both avian and human types, in their glands than did control animals; and the infection was still further increased by combining high humidity with the high temperature. Graziani (1906) studied the agglutinating power of the blood of rabbits kept at various temperatures. At 2° to 4° the blood would agglutinate at a dilution of 1 in 1541; at 18°, 1 in 854; at 32°, 1 in 727. In another series the blood of rabbits kept at 32° agglutinated at a dilution of 1 in 1250 while if the animals were occasionally relieved by cold baths the agglutinating power rose to 1 in 2425. Ritzmann (1907) kept guinea pigs, white rats and mice at 35° and found that heated animals died from half a day to three and a half days after injections of streptococci, control animals after one and a half to eight days. Injections of toxin-free tetanus spores and of tetanus spores plus streptococci yielded similar results. Ritzmann also cites experiments of Wysso-kowitsch leading to the same conclusion. Finally Ruata (1909) kept guinea pigs at a temperature of 30° with a relative humidity

of 85–95 per cent. and injected them with doses of typhoid, paratyphoid, dysentery and colon bacilli and cholera spirilla which were not fatal for normal animals. All the guinea pigs thus treated died in 4–26 hours, while, of control animals exposed to the heat alone, without injections, 30 per cent. succumbed.

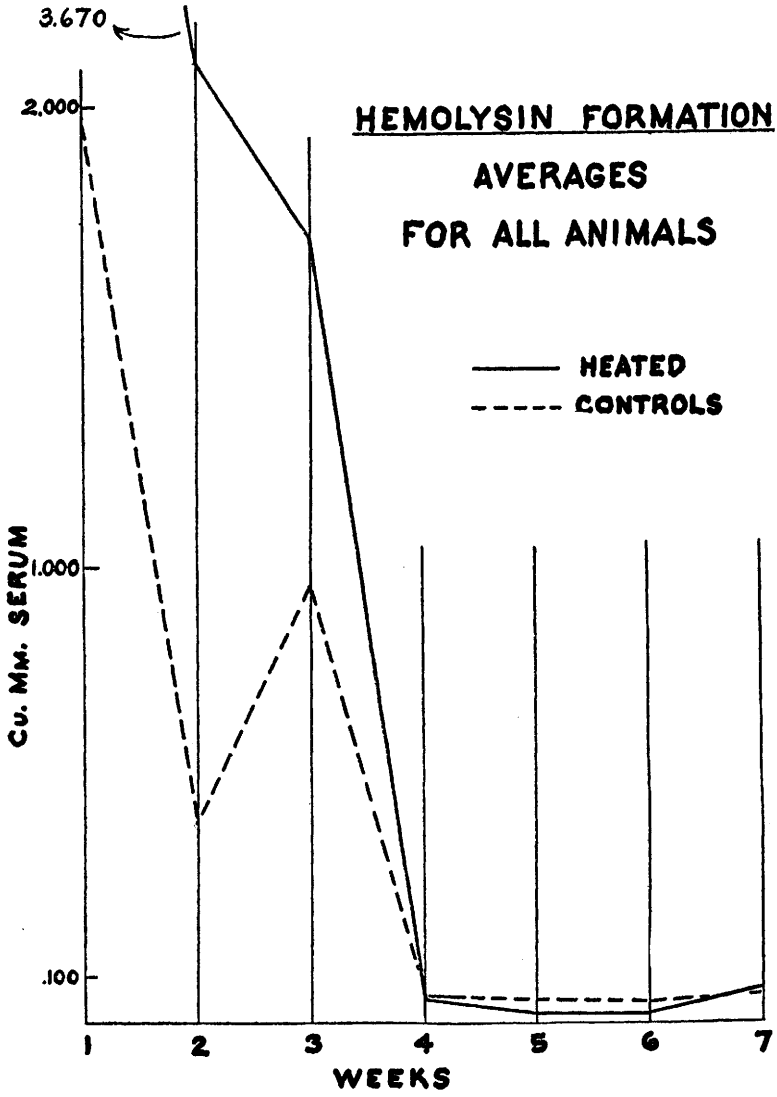

FIG. 1.

Our own experiments, which were undertaken as a part of the extensive studies of the New York State Commission on Ventilation, have dealt with this same problem of the effect of moderately high temperatures and were carried out in the bacteriological laboratores of the University and Bellevue Hospital Medical College.

Normal healthy rabbits were kept (2–4 at a time) in an incubator, 25″ x 48″ x 12′, at a temperature ranging from 29°–32° C. Control animals were kept at room temperature (18°–21°). At the beginning of the experiment each rabbit was bled (1 to 2 c.c.) and then inoculated intravenously with ½ c.c. of a 50 per cent. suspension of washed sheep erythrocytes. During the experimental period each rabbit was bled once a week for trial titrations of the hemolytic activity of the serum, and inoculations with the sheep cells in increasing doses were made twice a week during this period.

The hemolytic activity of the serum was determined as follows: The rabbit serum was inactivated at 55° C. for one half hour. A 5 per cent. suspension of sheep corpuscles was used, and for complement, normal guinea pig serum diluted 1–10.

The rabbit serum was prepared in varying dilutions, as indicated by the results of the previous titrations, and each dilution was then titrated in the same way.

A series of ten test tubes was set up with 0.1 c.c. of sheep corpuscle suspension and 0.1 c.c. of diluted guinea pig complement, and varying amounts of rabbit serum. The test tubes were then placed in a water bath at 37° for 1 hour. At the end of that time readings were made, and the smallest amount of rabbit serum of the dilution which gave complete hemolysis was taken as the hemolytic unit. Thus, if .06 c.c. of a dilution of 1–500 was the smallest amount of serum giving complete hemolysis, then .06 of this solution was taken as the hemolytic unit (.06 x 1/500 = 1/8333 c.c. = .120 cubic millimeters) and this decimal, representing the actual dilution of serum in cubic millimeters found effective under the conditions of the experiment, was taken as the measure of the hemolytic power of the serum. The figures in the table (used as ordinates in the chart) are derived in this way. 10 in the table means that 1/100 of a c.c. of serum (10 cubic mm.) showed no hemolytic activity. The error introduced into the

calculation of averages by calling this figure 10 when we only know that it was greater than 10 will not materially affect the results. Titrations were not performed during the second week of Series II. Series I and III were stopped after six weeks, Series V after five weeks and Series IV after four weeks. Other blanks in the table are due to the death of the animals.

Series.	Rabbit.	Air Temperature.	Hemolytic Power of Serum. (Cubic Millimeters of Serum Necessary to Hemolyze.)						
			1 Week.	2 Weeks.	3 Weeks.	4 Weeks.	5 Weeks.	6 Weeks.	7 Weeks.
I	1	30°	10.000	.118	.070	..	..	..	
	2	30°	.500	.083	.070	.059	.044	.022	
	51	20°	10.000	.161	.073	..	..	..	
	52	20°	10.000	.069	.050	.060	.105	.089	
II	186	30°	.145	..	.069	.020	.040	.047	.067
	187	30°	.100	..	.036	.034	.032	.054	.084
	183	20°	.178	..	.075	.014	.040	.055	.067
	184	20°	.189	..	.024	.040	.020	.033	.061
III	70	30°	.588	.060	.028	.025	.029	.033	
	75	30°	1.000	.067	.042	.022	.033	.040	
	86	20°	.213	.050	.024	.020	.025	.029	
	110	20°	.500	.067	.029	.017	.022	.028	
IV	88	30°	.588	.075	.020	.026			
	171	30°	.588	.069	.025	.032			
	4	20°	.500	.027	.020	.026			
	191	20°	.200	.044	.015	.020			
V	72	30°	10.000	10.000	.172	..	..		
	77	30°	10.000	.100	.164	..	..		
	100	30°	10.000	10.000	10.000	.200	.044		
	173	30°	.588	.400	10.000	.063	.044		
	83	20°	.123	.062	..	..	..		
	136	20°	.238	.270	10.000	.238	.083		
	147	20°	.833	.097	.076	.110	.083		
	148	20°	.588	.588	.161	.047	.040		
General average .		30°	3.670	2.100	1.720	.053	.038	.039	.076
		20°	1.970	.144	.958	.059	.052	.047	.064

The results as presented in the table appear to indicate a distinct decrease in the rate of hemolysin formation on the part of the heated rabbits. The hemolytic power of the blood of individual animals of course varies within wide limits, yet the averages show that in order to produce hemolysis it was uniformly necessary to use larger quantities of serum from the heated rabbits during the first three weeks. The influence of heat appears to show itself in a delayed formation of hemolysins rather than in a

permanent inhibition, as later on the average curves for heated and control animals are essentially the same. In Series III there were only two occasions in which the lowest serum strength for a heated rabbit fell below the highest for a control rabbit; and in Series IV, not one.

The wide variations exhibited in individual animals preclude the possibility of drawing definite and final conclusions from these results, but their general tendency, as evidenced by averages, agrees with the results of the other observers cited and they strongly suggest that moderately high air temperatures (30°) do not favor the development of immune bodies in the blood as higher temperatures producing a condition of fever have been reported to do, but on the other hand may be distinctly inimical to such development.