SUSCEPTIBILITY AND RESISTANCE TO TRYPANOSOME INFECTION.

II. The Relation of Physical Environment to Host Susceptibility to Infection.

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INTRODUCTION.

In this series of investigations, we set out to examine the relation of various factors to the resistance or susceptibility of experimental animals to infection with a protozoan—the trypanosome. In previous communications (1, 2) we have reported data bearing on the acquired susceptibility and resistance resulting from the interaction of the parasite and host. In this paper we present our observations on the effect of external environmental factors, as for example, light and moisture, on the susceptibility of the host to the infection and on the severity of the disease imposed.

Recent experimental epidemiologic studies by Webster (3) indicate that the virulence of a given strain of pathogenic organisms is constant. In that case variations in malignancy and host susceptibility can only be accounted for by assuming that the resistance of the host is variable, and is modified from time to time by a variety of conditions. Since many diseases have a seasonal character, this variation in resistance must have a seasonal rhythm.

Seasonal variations in the incidence and intensity of malaria outbreaks are noted everywhere. The seasonal occurrence of malaria epidemics is due to the optimum conditions for the development of the anopheline vectors. But, the relatively high prevalence of relapses with the advent of the spring and the occurrence of benign tertian malaria in the summer and of malignant tertian malaria in the fall still baffle the malariologists and await explanation. The questions still unsolved are: Whether the difference in the observed malignancy is due to

the greater or lesser virulence of the parasites, or to greater or lesser susceptibility of the host. If the latter is the case, what factors are responsible for the seasonal variation?

Recent experimental studies indicate a seasonal variation in the susceptibility of animals to certain diseases other than malaria. Brown and his associates (4) have shown that there is a seasonal rhythm in the malignancy of a transplanted tumor in rabbits, which the authors attribute to fluctuations in the resistance of the animals rather than to variations in the malignancy of the tumor. Pritchett (5) has shown a similar seasonal fluctuation in experimental mouse typhoid. The mortality among infected animals showed a distinct seasonal variation, being high in the spring and fall and low during the summer.

The causative factor in this seasonal variation of malignancy is not known. Lenz (6) attributes the increase of malaria relapses to sunlight, and puts forward the hypothesis that the sunlight stimulates parthogenesis of the gametocytes. There is no evidence in support of his hypothesis. Reinhard (7) presents evidence on the provocation of malaria relapses by ultra-violet light. Similar results are reported by Whitmore (8) in bird malaria. Brown and his coworkers (4) correlate the malignancy of the transplantable rabbit tumor with meteorological factors, low malignancy being associated with periods of maximum and minimum sunlight, while high malignancy occurred at times of sudden changes in sunlight both in the spring and autumn. Pearce and Van Allen (9) tested this observed correlation experimentally, and reported confirmative evidence. They found that animals kept in constant darkness showed a slight but distinct increase in the resistance to the tumor, while the effect under conditions of constant light was even more marked, the disease assuming a milder form.

It appears from the above that among the various environmental factors influencing host resistance light plays a significant rôle. But there are other environmental factors, among them heat and cold, dryness and moisture which may also play a part in determining host resistance. In this paper we present experimental data on the effect of light, darkness, and moisture on the resistance of guinea pigs to trypanosome infections.

Materials.

The strain of trypanosome used was *Tr. evansi* isolated from infected mules in 1923. Since its isolations this strain has been maintained in guinea pigs, and at present its virulence and pathological effects are quite constant. The strain invariably produces a fatal infection in guinea pigs and rabbits. In guinea pigs of 250 to 450 gm. the incubation period is quite constant, varying from 5 to 8 days with an average of 6.2 days. (Among 52 normal animals observed during 1925, the incubation periods were: six, 8 days; eleven, 7 days; twenty-two, 6 days; and

thirteen, 5 days.) The intensity of the infection as measured by the frequency of blood invasion also appears to be fairly constant. The duration of illness (or life) shows a tendency to seasonal fluctuations, a fact that will be discussed later.

Variations in the dosage of trypanosomes were not important within the limits used. Our dose was 0.1 cc. of citrated infected blood, diluted to contain an average of one organism per microscopic field, injected into the peritoneal cavity. In some of the experiments a counted number of organisms were injected (in one experiment 75 and in another 400), but no differences in the results were observed.

In so far as possible, guinea pigs of the same weight were used for each experiment.

Methods of Study.

The details of the methods used in each type of experiment will be described below. In general the animals used in a given experiment were first examined and then subjected to the conditions of the experiment for some days prior to the infection. The changes due to the infection as such could, therefore, be distinguished from those produced by the exposure to light, darkness, or moisture.

The difficulty in these experiments was to select a suitable criterion for decreased or increased susceptibility. The variations in individual animals render it difficult to rely on a single pathological manifestation. The length of the incubation period is the most constant phenomenon; but the frequency and intensity of the blood invasion and the duration of the illness are also of value. Consequently, these three have been employed as indices of the changes in host susceptibility produced by the exposure under the experimental conditions. Normal animals kept under the usual light conditions served as controls.

The first objective was to ascertain whether exposure to one or another of the conditions to be tested produced a measurable change in the host susceptibility. The experiments were wholly qualitative in character and the procedure fairly simple. Guinea pigs were exposed for various lengths of time to the action of direct sunlight nnd an equal number of comparable controls were kept in total darkness, or under ordinary light conditions. Under the conditions of the experiments sun and heat action were not separable, and in order to eliminate the heat factor, sets of animals were exposed to direct sunlight while immersed in water baths.

EXPERIMENTAL.

Preliminary experiments were made in order to ascertain the effects of the exposure under the various conditions of the experiments on the temperature, white cell, and differential counts of the animals. The results of these preliminary experiments are of interest in that they indicate that profound changes of greater or lesser duration may be produced by relatively short periods of exposure.

Exposure to Direct Sunlight.

An area of 4 to 5 cm. square was shaved on the back of animals which were then placed in a battery jar, or a wooden box. The jar or box was so inclined that the sun rays played constantly on the shaved area. In later experiments the animals were tied on an inclined board, and covered with a heavy towel, leaving only the shaved area exposed to the sun's rays. The exposure was always made between 9 and 9.30 a.m. with the sky and atmosphere clear. The facts must be emphasized that the experiments were carried out in Palestine, a region where the sunlight is of such great intensity that exposure of the guinea pig for but an hour may cause its death.

The exposure under water was carried out by the second of the methods described. The animals were tied to a board set obliquely in a tin box, made especially for the purpose, in such wise that the heads of the animals were outside the box. The heads were covered with a moist towel, and the box filled with water so that the shaved surface was 1 to 1.5 cm. under water.

After the exposure, the animals were placed in dry boxes in the animal room, and kept under the usual light conditions.

The effects of these exposures are illustrated in Tables I to III. An exposure of 15 minutes to direct sunlight raises the rectal temperature 1.5°C. or more, and changes the leucocytic formula. The temperature effect is temporary, lasting about 2 hours, but the blood cell change is more enduring, and recovery is very slow.

Insolation through a water bath produces none of these changes. The rectal temperature falls about 2.5°C., the blood cell formula remains unchanged, and there is an increase in the total leucocyte count. Even the infection, which is usually accompanied by a depression of the polynuclears prior to the invasion of the circulation, does not produce this effect in water-immersed animals until several days after the trypanosomes invade the circulation.

TABLE I.

Effect of Insolation on Normal Guinea Pigs.

Time of exposure 15 min. daily.

	Before exposure.	After exposure, *days*.						Increase on exposure.
		0	1	3	6	8	10	
Temperature	38.3°C.	39.9°C.	38.5°C.					1.5°C.
Total white cell count	10,600		9,600	8,900	9,000	8,880	9,600	
Polymorphonuclears	57%		48%	45%	47%	50%	51%	
Lymphocytes	37 "		47 "	49 "	47 "	45 "	46 "	
Large mononuclears	6 "		5 "	6 "	6 "	5 "	3 "	

Effect of 1 hr.'s exposure every day.

	Before exposure.	1	2	3	4	5	6
Total white cell count	8,800	8,600	8,800	6,400	7,900	9,000	
Polymorphonuclears	64%	56%	21%	40%	60%	55%	
Lymphocytes	35 "	42 "	78 "	58 "	38 "	43 "	
Large mononuclears	1 "	2 "	1 "	2 "	2 "	2 "	

Effect of ½ hr.'s exposure once.

	Before exposure.	1	3	4	5
Total white cell count	9,000		6,800	7,800	8,400
Polymorphonuclears	58%	40%	54%	51%	57%
Lymphocytes	39 "	58 "	42 "	46 "	41 "
Large mononuclears	3 "	2 "	4 "	3 "	2 "

Effect of 1 hr.'s exposure once.

	Before exposure.	1	2	3
Total white cell count	8,600	9,200	8,000	7,800
Polymorphonuclears	57%	36%	45%	47%
Lymphocytes	40 "	61 "	53 "	50 "
Large mononuclears	3 "	3 "	2 "	3 "

The preliminary experiments showed that exposure to the conditions described, even though of short duration, produced appreciable

TABLE II.

Effect of Insolation through Water on Normal Guinea Pigs.

Time of exposure 15 min.

	Before exposure.	After exposure, *hrs.*			
		0	2	4	24
Temperature	38.9°C.	36.2°C.	38.3°C.	38.6°C.	38.9°C.
Total white cell count	9,800		10,600	12,000	11,000
Polynuclears	58%		56%	56%	59%
Lymphocytes	36 "		38 "	39 "	35 "
Large mononuclears	6 "		6 "	5 "	6 "

TABLE III.

Effect of Insolation, Direct and through Water, on the Blood Picture and Blood Infection.

Time of exposure 15 min.

After infection.	Direct.					Through water.					Control.				
	Total differential count.				Tr.[1]	Total differential count.				Tr.[1]	Total differential count.				Tr.[1]
	W.c.c.	P.	L.	M.		W.c.c.	P.	L.	M.		W.c.c.	P.	L.	M.	
days															
1	9,600	50	45	5	0	12,800	56	39	5	0					
2	8,900	47	49	4	0	14,000	54	40	6	1:5	8,000	50	45	5	0
4	8,200	45	50	5	1:10	14,800	52	44	4	3:1	7,900	45	51	4	0
5	7,200	43	52	5	1:5										0
6						11,000	48	48	4	2:1	7,000	41	54	5	1:5
7						12,400	47	47	6	6:1					

[1] Number of trypanosomes per microscopic field.

changes in the course of the infection. Exposure to direct sunlight for 15 minutes shortened the incubation period to 3 days instead of the 6 of the controls, while in the animals insolated under water for

the same period, the incubation period was only 2 days, and there was a prompt and heavy invasion of the blood stream.

The duration and time of exposure make an appreciable change in the results in animals infected with the trypanosome. A single exposure of half an hour on the day of infection reduces the incubation period, and shortens the duration of the illness by about half. A longer exposure, 1 hour or more, may, as already stated, cause sudden death with acute symptoms: the exposed area is inflamed, there is severe hemorrhage in the peritoneal cavity, the coagulability of the

TABLE IV.

Effect of Insolation on the Course of Trypanosome Infection in Guinea Pigs.

(a) Insolated directly.						(a) Insolated through water.					
No. of animals.	Length of exposure before infection.	Time of exposure.	Average incubation period.	Frequency of blood invasion.	Average duration of illness.	No. of animals.	Length of exposure before infection.	Time of exposure.	Average incubation period.	Frequency of blood invasion.	Average duration of illness.
	days	*min.*	*days*	*per cent*	*days*		*days*	*min.*	*days*	*per cent*	*days*
2	2	5	2½	67	60	3	2	5	2½	67	58
7	5	10	4¼	74	58	—	—	—	—	—	—
3	14	15	4	62	50	2	14	15	3½	67	56
(b) Kept in complete darkness.						(b) Normal light.					
2	2	Constant.	5½	48	71	4	—	—	6	55	69
7	5	"	8	53	91	8	—	—	6¼	56	85
3	14	"	7	40	99	4	—	—	6	50	79

blood is reduced, the spleen is enlarged, and the vessels of the abdominal viscera are distended with multiple capillary hemorrhages.

The more detailed subsequent experiments confirmed and extended these results. We studied, particularly, in a qualitative way, the effect on the course of the trypanosome infection of various degrees of exposure of immersed and non-immersed animals to direct sunlight. The results are summarized in Table IV, *a*. The course of infection in controls kept under ordinary light conditions or in total darkness is shown in Table IV, *b*.

Although the results are only qualitative in character, they indicate

clearly that the animals kept in ordinary diffused light and especially those kept in total darkness were more resistant to the infection than those exposed for brief periods daily to the direct action of sunlight.

We have made some attempts to gauge the intensity of the sun rays by the Clark method (10). The first standard furnished through the kindness of Dr. Clark was not satisfactory. The second was more so, but it was received after our experiments were well under way. A comparison of intensities was not possible therefore. With the second standard an 8 minute exposure equalled one lithophone unit (50 per cent reduction).

The peculiar effect of immersion on the course of infection, in the absence of any change in the leucocytic ratio, led us to test the influ-

TABLE V.

Effect of Immersion of Animals for Short Periods in Water on Their Resistance to Trypanosome Infections.

No. of animals.	Time immersed.	Incubation period.	Frequency of blood invasion.	Duration of life.	Remarks.
	min.	*days*	*per cent*	*days*	
5	15	3.4	86	54	Both sets of animals were kept under the same conditions except for the immersion in water.
5	0	6	63	82	

ence of immersion for short periods without radiation. The results were practically identical with those observed in radiated immersed animals. It appears, therefore, that immersion as such produces a profound change in the resistance of guinea pigs to trypanosome infections. This has been noted in a number of experiments and cannot, therefore, be considered accidental.

It remains to be determined whether the effects produced by short exposures to direct sunlight are due to heat or specific rays and whether those produced by immersion are due to chilling or excessive moisture of the skin. It is clear, however, that the exposure of infected animals for brief periods daily causes significant changes in the course of the infection.

The lowering of resistance to trypanosomes which follows upon

immersion in water or exposure to sunlight for short intervals may be referable to the same causes which are responsible for the production of malarial relapses on exposure to light or chilling. The relatively great resistance shown by animals kept under conditions of complete darkness accords with the results reported by Pearce and Van Allen (9) in experiments with a rabbit tumor.

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