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Seasonal Variation of the Phagocytic Activity of the Reticulo-Endothelial System

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Summary. Observation of the phagocytic activity of the reticulo-endothelial system in the healthy male albino mouse extending over 24 months showed a significant seasonal variation. This was found to range from a high level in the summer to a low one in the winter. The degree of variation was found to be constant for particular seasons in succeeding years. Assessment of phagocytic activity was made by measuring the rate of absorption of colloidal carbon from the blood stream (Halpern, Benacerraf and Biozzi, 1953). The causes of the variation are attributed to environmental factors and the individual mechanisms are discussed. A monthly correction factor is suggested so that investigations of phagocytic activity at different times of the year may be compared. An extension of the correction factor is proposed so that the variations in phagocytic activity produced by various agents can be related to a standard value.

INTRODUCTION

In previous studies on the effects of chemotherapeutic agents on the phagocytic activity of the reticulo-endothelial system of the mouse (Nicol and Sewell, 1959; Sewell and Nicol, 1958), it was found that the same agent produced results which varied from one season to another, although standard conditions of investigation were employed in each case. It was also found that this seasonal variation was maintained over successive years. The present investigation was designed to determine the extent of the seasonal variation, and to devise a method of comparing results obtained at different times of the year. From these studies, a correction factor was sought whereby the effects of various agents on phagocytosis could be compared.

MATERIALS AND METHODS

Ninety male white mice (T.O. Swiss strain) aged 3 to 5 weeks were used in these experiments. Body weight ranged from 18 to 25 g. They were fed on a standard diet of Oxoid Diet 4 lb. modified pellets (Short and Parkes, 1959; Bruce, 1950). Drinking water was readily available from water containers in the cages. Thermal control of the air temperature in the animal house depended on a regulator which switched on when the temperature fell below 15°, and heating was cut off when the temperature rose above 20°. The only time the animals were handled was for cleaning daily by the animal house attendant. Each group of animals was observed for periods of 8 to 10 days.

The method used to assess reticulo-endothelial activity was that originally described by Halpern, Benacerraf and Biozzi (1953) in which a known amount of colloidal carbon was given intravenously via the dorsal vein of the penis and the rate of its disappearance from the blood stream was assessed photometrically by an E.E.L. absorptiometer with a red filter. The size of the carbon particle used was 250 Å, an optimum size for the least amount of intravascular agglutination and precipitation. The amount of carbon given

was calculated on the basis of 16 mg. of dry weight of carbon per 100 g. mouse body weight. The logarithmic values of the absorptiometer readings gave an exponential curve, the tangent of which was proportional to the phagocytic index. The index was given the symbol K and is 100 tan a.

The animals were studied in groups of five. They were killed by stunning and exsanguination so that autopsy 1 hour after administration of the carbon could be carried out to assess the liver and spleen morphologically and microscopically. These two organs contain more than 90 per cent of the active reticulo-endothelial tissue in the mouse.

The investigations extended over 24 months.

TABLE 1

MONTHLY RECORDS OF PHAGOCYTIC INDEX (K VALUES) IN NINETY MICE OVER A PERIOD OF 24 MONTHS

Year	*Jan.*	*Feb.*	*Mar.*	*April*	*May*	*June*	*July*	*Aug.*	*Sept.*	*Nov.*	*Dec.*
1957					21	20		20		13	
					23	26		21		14	
					24	28		22		14	
					26	29		24		14	
					27	31		25		15	
					24 ± 2.4	27 ± 4.2		22 ± 2.4		14 ± 1.0	
1958	11	10		18	22	20	19	16	13	10	10
	12	12		18	24	22	19	19	15	11	13
	13	13		20	26	23	20	20	16	12	14
	13	15		21	28	24	23	23	16	12	14
	14	16		24	30	26	24	24	17	14	17
	13 ± 1.2	13 ± 1.4		20 ± 2.5	26 ± 3.2	23 ± 2.2	21 ± 2.4	20 ± 3.2	15 ± 1.2	12 ± 1.1	14 ± 1.4
1959	10		16	16	22						
	13		16	18	23						
	13		18	18	23						
	13		19	21	24						
	15		20	22	28						
	13 ± 1.1		18 ± 1.3	19 ± 1.6	24 ± 1.4						
Mean m'n'ly K value	12.7	13.2	17.8	19.6	24.8	23.8	21.2	21.4	15.4	12.8	13.6

RESULTS

Table 1 records the K values obtained for all animals during the period of investigation. The mean monthly K values over 24 months are given in Fig. 1 which shows that the K values were lower in the winter than in the summer. For statistical purposes the mean K value during the summer months was compared with that for the winter months, and the difference was found to be highly significant (Table 2). The monthly rhythm in K value was approximately the same over 2 successive years and a correction curve was obtained by plotting the mean monthly K values (Fig. 1).

Only the results from healthy animals have been included in the investigation. The rate of growth during observation was from 1.5 to 2.5 g. per week.

DISCUSSION

Seasonal variation in reticulo-endothelial activity in the mouse may be a reflection of seasonal variation in immune response, for it is known that the seasonal levels in immune bodies vary from one time of the year to another in both man and animals (Junge and Rosenthal, 1948). In clinical studies in man, it has been established that infective illnesses

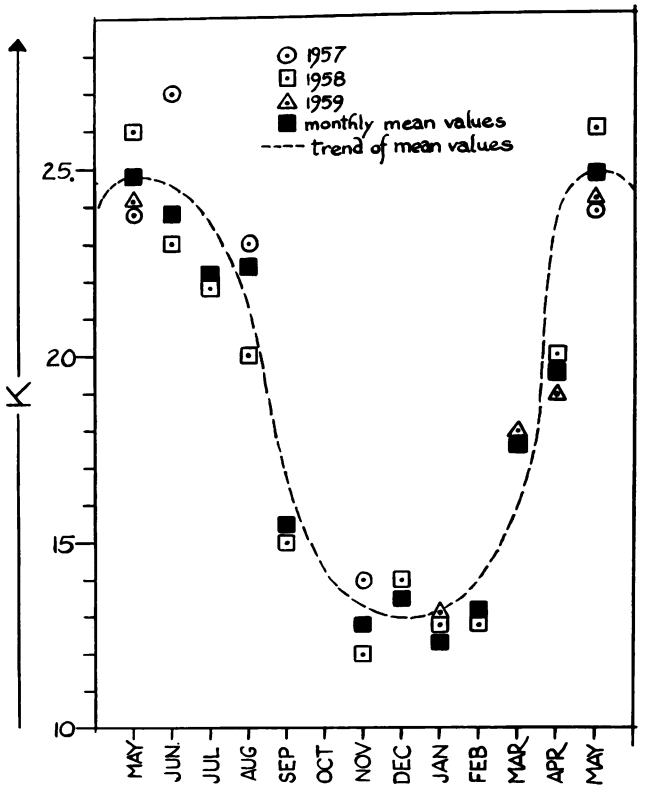

FIG. 1. Seasonal variation in phagocytic activity. Monthly K values for 24 months.

TABLE 2

STATISTICAL COMPARISON OF MEAN K VALUES DURING SUMMER AND WINTER MONTHS

Mean K value (May–August)	24 ± 3.4	$p < 0.001$
Mean K value (November–February)	14 ± 3.0	

are more common in winter, while their severity falls in summer (Ryle, 1948); and recent work by McKelvie and Nicol (unpublished) in this laboratory has shown that the reticulo-endothelial system is actively involved in acute inflammatory processes. The fact that the incidence of acute inflammation is greater in winter than in summer is probably related to a seasonal variation in the activity of the reticulo-endothelial system.

The factors which cause seasonal variation are likely to be air temperature, humidity or sunlight or a combination of all three. These are the factors which could not be

accurately controlled in the observations, and there is good experimental evidence to support these factors as possible causes.

The temperature of the animal house may have had some effect on the phagocytic activity because the peak of this activity coincided with the higher temperatures of summer, and the low levels of activity were related to the lower temperatures of winter. So far, there appears to be no simple explanation of the effect of temperature change on phagocytosis, and a repetition of this type of experiment in a thermostatically controlled air-conditioned laboratory would be interesting. It is relevant that Ipsen (1952) found that mice injected with tetanus toxoid produced minimal amounts of antitoxin if maintained at a temperature of 6°, but gave increasingly better antitoxin responses at 25° and 35° respectively.

The humidity of the air of the animal house varied with that of the external air, since no air-conditioning was in operation during the present investigations. Variations in humidity cause reflex changes in the peripheral vascular system as part of a general body response (Winslow, Harrington and Gagge, 1937), and this may have been a factor in mobilizing macrophages from an inactive state in the peripheral capillary system to an active one in the circulation (Jaffe, 1938).

Ultraviolet radiation of skin produces an erythema (Wright, 1952) which is due to capillary dilatation of existing and latent capillary networks and may have enhanced the mobilization of previously inactive macrophages, to which process also, humidity may have contributed.

The present research by the carbon method has shown that for each month there is a particular level of phagocytic activity in the normal mouse and that a correction curve can be obtained by plotting the mean monthly K values. It was also found in the mouse that antibiotics and sulphonamides produce the same order of variation in phagocytic activity at different times of the year, and it is suggested that reference to the monthly correction curve (Fig. 1) could be made to compare the effects of therapeutic agents on phagocytosis at different times of the year. It is proposed that the vertical distance between the normal K value for the month concerned and the K value obtained for the therapeutic agent during the same month be given a positive or negative value according to whether it is greater or less than normal. This difference could be recorded as $+cK$ or $-cK$, where cK is the corrected K value. If the cK values for other times of the year are known, then an overall cK value can be calculated as the arithmetic mean of the known cK values. In addition, the extent of the variation may be calculated as follows:

$$\text{Extent of variation} = (cK \text{ maximum} - cK \text{ minimum})/cK \text{ mean}$$

It is suggested that the extent of the variation be given the symbol cKv.

It should be emphasized that the monthly correction curve will vary from one laboratory to another, the correction being minimal in those with effective air-conditioning.

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