

too was stimulatory. Despite these stimulatory effects, Exp. 14 demonstrated that the addition of such substrates will not alter a depressing effect between two sets of different but viable cells. Much more work is needed to elucidate the mechanisms involved in these observations.

Summary. 1. The uptake of S^{35} -L-methionine into the protein of pooled and non-pooled liver slices in guinea pigs has been studied. 2. Defining the mean of liver uptakes

from 2 animals as 100%, when the two guinea pig livers were pooled, an uptake of 58 to 66% was found. 3. No evidence for a diffusible inhibitor could be demonstrated.

1. Rutman, R., Dempster, E., and Tarver, H., *J. Biol. Chem.*, 1949, v177, 491.

2. Canzanelli, A., Rapport, D., and Guild, R., *J. Biol. Chem.*, 1950, v183, 291.

3. Ranney, H. M., and London, I. M., *Fed. Proc.*, 1951, v10, 652.

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Changes in Eosinophil Count of Mice with Venisections* Repeated at Intervals of Several Days.† (20053)

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In the course of studies on characteristic eosinophil levels(1) it was noted that the mean eosinophil count of groups of inbred mice obtained at four-day intervals showed marked differences from one sampling time to the other. Such results were obtained despite the observance of certain precautions to control the physiological eosinophil rhythm(2). These included 1) not only the procedure of sampling at the same times of day, but 2) the equally relevant standardization of experimental animals by means of a regimen of single housing for at least nine days in an air conditioned room at the temperature of $78 \pm 2^\circ\text{F}$ with standardized lighting (light from 06:00 to 18:00 and darkness from 18:00 to 06:00)‡ and *ad libitum* feeding.

The methods of handling and counting have been described(1). The counts were made at

the times indicated in the tables. The periods chosen are during the rising phase of the usual daily rhythm. It is noted that 2 counts were obtained on each of the days of observation at an interval of 4 hours. The first of the 2 counts for each animal and for each day of observation is presented.

A summary of the data on eosinophil counts obtained under these circumstances is presented in Table I. It is apparent that the mean count obtained for the third venisection is lower than that obtained for the first venisection for each of the 10 stocks of mice investigated.§ It appeared undesirable to accept these observations as representative of a more general phenomenon in mice, since the data were obtained from very homogeneous populations of animals. It does not follow *a priori* that results valid for particular stocks and circumstances would necessarily be valid for other biological material, even though closely related. Yet it appears that they are valid.

* Although the term venisection is employed for linguistic convenience it is recognized that the samples of blood obtained from a small incision in the mouse's tail were either venous or arterial, or mixed. For the purposes of this investigation it is believed that the distinction between venous and arterial blood is not significant.

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‡ Standardized environmental circumstances, especially controlled lighting, appear to be necessary to assure constancy of the temporal placement of physiological eosinopenia within the 24-hour period (3).

§ These mice have been described(1).

TABLE I. Mean Eosinophil Levels in Three Successive Determinations at 4-Day Intervals on Several Stocks of Mice.

Stock	No. of animals	Eosinophils/mm ³ of tail blood*	
		Median	Mean
B ₁	17	969	1022
	14	825	1022
	"	750	684
B ₄	15	687	700
	14	675	661
	"	594	580
D ₈	"	259	297
	"	212	195
	"	119	142
ZD ₈ F ₁	"	125	130
	"	131	129
	"	87	83
Z	"	181	179
	"	150	176
	"	106	119
Z _B	"	169	198
	15	106	113
	"	75	91
A	14	175	166
	"	75	112
	"	75	85
A _X	"	162	168
	"	100	100
	"	87	81
Z _B A _X F ₁	11	131	143
	"	100	115
	"	112	125
A _X Z _B F ₁	10	112	129
	"	119	113
	"	94	110

* Counts obtained from 06:00 to 10:00.

Comparable data from a larger group of genetically less homogeneous mice are presented in Table II. Individual counts are listed to illustrate the variability of the changes in individual animals. The general trend, however, is again a slight but progressive decrease in number of eosinophils from one sampling time to the other.

Table III summarizes the results of an analysis of variance applied to these data. In the presence of the expected significant inter-individual differences the difference in mean eosinophil level between the successive experiments is also significant below the one per cent level (the F value required for the 1 per cent level is 3.48; that obtained for the differences between successive experiments is 7.24). Whatever may be the cause of these differences, it appears obvious that the changes are not simply intra-individual random variations.

Rather the past history of a mouse—including venisections performed several days prior to an eosinophil count—has a definite effect upon the number of circulating eosinophils.

Two approaches are practicable for control of factors like these venisections in the experimental analysis of the effects of a given

TABLE II. Five Successive Eosinophil Counts on 2-Month-Old Male ZBC* Mice, 44 Mice.

	Eosinophils/mm ³ tail blood—successive determinations†				
	1	2	3	4	5
	42	39	36	22	17
	36	28	45	58	45
	55	78	62	73	50
	87	100	139	111	83
	226	139	92	92	75
	126	97	51	125	42
	55	47	222	239	76
	396	81	67	41	39
	207	246	37	70	303
	101	62	133	55	81
	83	129	98	84	69
	106	147	76	58	58
	181	172	89	83	129
	80	128	92	33	47
	87	69	78	42	44
	69	53	33	36	22
	70	69	28	47	44
	61	72	105	20	28
	27	66	117	51	42
	28	92	83	64	37
	133	158	72	84	95
	204	167	23	41	33
	87	103	87	34	28
	123	34	22	20	33
	23	42	103	9	17
	148	142	109	114	129
	262	193	220	195	80
	117	140	37	78	80
	53	204	173	172	151
	279	47	254	133	181
	94	86	150	120	30
	103	66	105	34	31
	133	289	98	67	78
	19	58	183	73	41
	285	168	81	62	62
	238	129	105	98	53
	198	162	140	66	56
	253	129	92	90	45
	31	87	61	62	90
	39	76	159	198	144
	173	222	184	158	131
	75	55	44	22	58
	39	53	105	112	28
	253	217	131	125	165
Exp. mean	125	112	101	81	72

* The fostered A and Z stocks are mated to produce either the A_XZ_BF₁ or the Z_BA_XF₁ hybrids. The progeny of F₁ females backcrossed to Z_B males is referred to as ZBC.

† Counts obtained from 02:00 to 06:00 at 4-day intervals.

TABLE III. Summary of Analysis of Variance.

Source of variation	D.F.	Var- iance	F	F. _{.05}	F. _{.01}
Between mice	43	99122.62	3.13	<1.50	<1.76
Between exp.	4	21104.88	7.24	<2.45	<3.48
Pooled residual	172	2914.11			

variable. An experimental design providing for all possible protection of experimental animals against extraordinary stimulation has great merits. Animals with no past history of venisection, *e.g.*, can be used whenever comparable populations of experimental animals are available. Statistically significant groups of animals can then be studied at several time points if need be. However, the other possible investigative design, involving the use of animals exposed to the same number of venisections, is often a necessary concession to practicability. It is recognized, however, that this second procedure is open to some criticism. It would approximate control of the stimulation only if mice subjected to venisection could be handled identically. And even if this were feasible it would not necessarily follow that two groups of experimental mice will react identically to venisection and/or the handling associated with this operation.

If eosinophil counts are to be dealt with as a quantitative variable, control of the past history of the animals employed will have to be established, in addition to scrupulous care

for the maintenance of standardized environmental(3) and temporal(2) circumstances. It is conceivable that effects on this variable could then be evaluated as effects on a fundamental physiological cycle in mice(4) and in man(5,6).

Summary. The numbers of circulating eosinophils in the tail blood of mice have been determined at intervals of several days. A decreasing trend in mean number of eosinophils is noted with repeated venisections for various stocks of mice. The procedural implications of the finding are discussed in the light of related data. Control of the past history of experimental animals appears to be necessary if eosinophil counts are to be evaluated quantitatively.

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1. Halberg, F., Bittner, J. J., and Visscher, M. B., *Blood, J. Hematology*, 1951, v6, 832.
2. Halberg, F., and Visscher, M. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1950, v75, 846.
3. ———, *Am. J. Physiol.*, in press.
4. ———, *Endocrinology*, in press.
5. Halberg, F., Visscher, M. B., Flink, E. B., Berge, K., and Block, F., *J. Lancet*, 1951, v71, 312.
6. Halberg, F., *J. Lancet*, 1953, v73, 20.

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Degenerative Forms of Eosinophilic Leukocytes in Lymphoid Organs.* (20054)

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Recent reports have dealt with the fate of the eosinophilic leukocyte following various stimuli which reduce their numbers in the peripheral blood stream. Padawer and Gordon (1,2) have described the process of breakdown

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of eosinophils in peripheral blood and in the pleural and peritoneal fluids of rats treated with epinephrine or cortisone. It has also been reported(3) that significant decreases occur in the numbers of eosinophils following their incubation *in vitro* with cortisone. The present report provides evidence that eosinophils are found normally in varying stages of breakdown in lymph nodes and that lowered