2. Levin, M. H., Leo F., and Bassett, S. H., *J. Clin. Endocr. and Metab.*, 1952, v12, 506.

3. Robinson, W. D., Conn, J. W., Block, W. D., Louis, L. H., and Katz, J., *Rheumatic Diseases, Am. Rheumatism Assn.*, Saunders Co., Philadelphia and London, 1952, p. 241.

4. Wolfson, W. Q., Cohn, C., Levine, R., Rosenberg, E. F., Hunt, H. D., and Guterman, H. S., *ibid.*, p254.

5. Speirs, R. S., and Meyer, R. K., *Endocrinology*, 1949, v45, 403.

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Blood Clotting Time and Tissue Mast Cell Number of the Bat (*Myotis lucifugus*) in Different Physiological States. (21137)

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The bat is one of a number of mammals which in nature pass the winter in a state of dormancy characterized by low rates of respiration, heart beat, blood flow and oxygen consumption(1). Laboratory studies have shown that the resting bat is essentially poikilothermic(2,3) and that its rates of blood flow(3) and oxygen consumption(2,3) vary directly with the ambient temperature. At low temperatures (5-10°C) the red blood cells traverse the vessels in clumps and rouleaux, but thrombi and stoppage of flow are not seen(3). At high temperatures (20°C and upward) blood flow is much like that found in other mammals. Since intravascular clotting and thrombus formation do not accompany the sluggish circulation of the dormant bat, we have tried to establish whether different blood clotting times characterize the profoundly different physiological states which may be induced in the bat. We have already noted(4) that clotting time is markedly prolonged when the summer bat is exposed to cold. The present paper is concerned with the extension of these studies to winter bats and with the possibility that changes in clotting time might be accompanied by changes in the tissue mast cells which are thought to store and release the anti-coagulant, heparin(5,6).

Methods. *Small brown bats* (*Myotis lucifugus*) were collected in the winter from a cave in northern Illinois and in the summer from the attic of a farm house in southern Indiana. Blood clotting times were determined (in all cases at 23°C) by the capillary tube technic on samples obtained by puncturing a large vein in the web between the hind legs and from the cut carotid artery (the clotting time of the blood from each source was essentially the same). In each bat counts were made of the number of mast cells in single 12 μ thick sections of duodenum magnified 264x. The tissues were fixed in absolute alcohol and stained with toluidine blue. Studies were carried out at the time of collection and later in the laboratory at various times after placing summer bats in a refrigerator (5°C) and winter bats in an air conditioned room (23°C).

Results and discussion. In nature markedly different clotting times distinguish the summer bat from the winter bat (Table I). That of the former is short as in the homoiothermic mammals, while that of the latter is very long as in the aestivating ground squirrel(7) and in the hibernating hedgehog(8) and hamster(9). We interpret these data to indicate the existence of an adaptive mechanism which (a) prevents thrombus formation under the conditions of slow blood flow found in the dormant winter bat and (b) allows for rapid blood coagulation in the active summer bat. Table I also indicates that such a mechanism operates upon exposure of summer and winter bats to different ambient temperatures. Summer bats become dormant upon exposure to cold and show a marked prolongation of clotting time, while dormant winter bats awaken upon exposure to 23°C and manifest

TABLE I. Blood Clotting Time and Tissue Mast Cell Number of the Bat in Different Physiological States.

Experimental conditions	No. of bats	Blood clotting time (min.) Min	Max	Avg	Mast cell No. (avg)
Summer bat (on collection)	10	1.5	24	7.4	121
Winter bat (" ")	13	45	>360	(180)*	119
Summer bat					
On 16th day at 5°C	4	72	275	149	152
" 50th " " 5°C	2	170	210	190	169
Winter bat					
After 60 min. at 23°C	4	2	11	5	—
On 1st day at 23°C	9	5	26	12	50
" 4th " " "	10	7	32	18	26
" 7th " " "	10	6	60	25	19
" 14th " " "	10	6	47	19	61
" 21st " " "	6	4	13	8	40

* Median.

a sharp decrease in clotting time. The mechanism is quickly called into play, since within an hour after bringing the dormant winter bat into a warm environment (23°C), the clotting time is reduced to that of the summer bat.

The mast cell data are also found in Table I. The tissue mast cell population of sections of duodenum is the same in the dormant winter bat as in the active summer bat. Upon exposure of the former animal to 23°C and the latter to 5°C there appear to be decreases and increases respectively in the mast cell content of the duodenum. The reduction of mast cells in the winter bat is statistically significant, while the increase in the summer bat is not. Since these changes and indications of changes in mast cell number are accompanied by alterations in the clotting time of the blood, it is possible that the mast cell alterations, in part at least, may account for the different clotting times. Thus increased numbers of mast cells are associated with an increased availability of heparin and prolonged blood clotting; while decreases in mast cells are associated with a lowered availability of heparin and rapid blood clotting. Similar changes have been observed in the hedgehog and have been given a like interpretation(8). Microscopic examinations of sections of the duodenum and of whole mounts of the skin gave no information on the fate of mast cells disappearing when the winter bat is placed at 23°C. The number of abnormal (degenerated)(10) mast cells was small and essentially constant in all the conditions studied. Failure to find differences in the absolute number of mast cells in duodenal sections from summer and winter bats might be accounted for by differences in strain.

Summary. 1. Blood coagulation times and tissue mast cell counts were determined on summer and winter bats and upon their exposure to 5°C and 23°C, respectively. 2. In nature the blood clotting time of the active summer bat is short, while that of the dormant winter bat is prolonged. Exposure of the summer bat to 5°C and the winter bat to 23°C in the laboratory results in a lengthening and a shortening of the clotting, respectively. The number of tissue mast cells in the duodenum appear to increase in the former case and decrease in the latter instance. 3. The above changes are interpreted as indicating the presence of a mechanism which prevents thrombus formation under the conditions of slow blood flow during dormancy and which allows for rapid clotting when the bat is active. The mast cell changes suggest that the alterations in clotting time are determined at least in part by alterations in the availability of heparin to the circulation.

1. Hall, M., in Todd, R. B., (ed.), *The Cyclopedia of Anatomy and Physiology,* London: Longman, Brown, Green, Longmans and Roberts, 1836, v2, 764.

2. Hock, R. J., *Biol. Bull.,* 1951, v101, 289.

3. Smith, D. E., and Svihla, G., unpublished observations.

4. Smith, D. E., Lewis, Y. S., and Svihla, G., *Experientia,* in press.

5. Holmgren, H., and Wilander, O., *Z. mikroskop. anat. Forsch.*, 1937, v42, 242.
6. Jorpes, J. E., *Heparin*, 2nd edit., Oxford University Press, New York, 1946.
7. Svihla, A., Bowman, H. R., and Ritenour, R., *Science*, 1951, v114, 298.
8. Suomalainen, P., and Lehto, E., *Experientia*, 1952, v8, 65.
9. Svihla, A., Bowman, H., and Pearson, R., *Science*, 1952, v115, 272.
10. Smith, D. E., and Lewis, Y. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 306.

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Inhibition of *in vitro* Heme Synthesis from N^{15}-Glycine by 2-Ethyl-5-methylbenzimidazole.*† (21138)

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Avian erythrocytes incorporate N^{15} into heme when incubated with N^{15}-glycine and provide an *in vitro* biological system for the study of heme synthesis(1). Because of the chemical relationships of methyl-substituted benzimidazoles to the benzimidazole moiety of vit. B_{12} and to the purines, we investigated the effect of their addition to this system(2).

2,5-Dimethylbenzimidazole previously had been noted to act as a growth depressant when fed to rats in tests of compounds for vit. B_{12}-like activity(3). It inhibited red blood cell formation in mice made anemic by phenylhydrazine injection(4) and inhibited influenza virus multiplication(5).

We found that 2,5-dimethylbenzimidazole and 5,6-dimethylbenzimidazole prevented incorporation of N^{15} from N^{15}-glycine into heme. Benzimidazole and 2-methylbenzimidazole at the same concentration had slight but not comparable activity. 5-Methylbenzimidazole had an inhibitory effect intermediate between that of benzimidazole and 2,5-dimethylbenzimidazole(2).

These compounds, in approximately the same concentration and in precisely the same relative order of inhibitory activity, were found by Tamm *et al.*(6) to inhibit multiplication of influenza A or B virus. The striking coincidence of relative inhibitory activities of the methyl-substituted benzimidazoles for 2 widely different systems, *in vitro* hemoglobin synthesis by avian erythrocytes and virus duplication, indicated to us that some fundamental mechanism underlying both processes might be involved.

Tamm *et al.*(6) found 2-ethyl-5-methylbenzimidazole to be a much more potent antiviral agent than any of the benzimidazoles studied by us in the avian erythrocyte system(2). It was considered to be approximately 7 times more effective than 2,5-dimethylbenzimidazole and 19 times more so than benzimidazole. It is thus of importance to know whether 2-ethyl-5-methylbenzimidazole likewise is a more effective inhibitor than 2,5-dimethylbenzimidazole for the synthesis of heme by the avian erythrocyte. This has been determined to be so in the present study and supplies additional support to the concept that the same fundamental mechanism underlies both processes.

Experimental. Fresh chicken blood was obtained for each experiment. Penicillin and streptomycin were added to inhibit microbial action during incubation(1). Twenty ml blood samples were incubated with the experimental compound and 25 mg N^{15}-glycine (31 atom % N^{15}) in 50 ml Erlenmeyer flasks with constant shaking for 24 hours in a water bath at 37°C. In each experiment the effect on

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