D-(−) muscarine and all racemic stereoisomers (epi, allo, epiallo) possess only a fraction of the potency of dl-muscarine. 3. Keto analogues of muscarine (muscarones) are more potent than muscarine in stimulating smooth muscle; in addition, they possess, on frog skeletal muscle, remarkable nicotinic activity of which the muscarines are almost devoid. 4. The potency of the muscarones is not stereospecific. Both + and − muscarones are more potent than L-(+) muscarine, and dl-allo muscarone is only slightly less potent than dl-muscarone.

1. Schmiedeberg, O., Koppe, R., *Das Muscarin*, F. C. W. Vogel, Leipzig, 1869.

2. Kögl, F., Duisberg, H., Erxleben, H., *Liebigs, Ann. Chem.*, 1931, v489, 1956.

3. Eugster, C. H., Waser, P. G., *Experientia*, 1954, v10, 298.

4. Eugster, C. H., *Helv. Chim. Acta*, 1956, v39, 1002.

5. Kögl, F., Salemink, C. A., Schouten, H., Jellinek, F., *Recueil*, 1957, v75, 109.

6. Kögl, F., Cox, H. C., Salemink, C. A., *Experientia*, 1957, v13, 137. Corrodi, H., Hardegger, E., Kögl, F., Zeller, P., *ibid.*, 138.

7. Hardegger, E., Lohse, F., *Helv. Chim. Acta*, 1957, v40, 2383.

8. Eugster, C. H., Häfliger, F., Denss, R., Girod, E., *ibid.*, 1958, v41, 705.

9. ———, *ibid.*, 1958, v41, 886.

10. ———, *ibid.*, 1958, v41, 205.

11. ———, *ibid.*, 1958, v41, 583.

12. Shingh, Grewal R., *Brit. J. Pharmacol.*, 1951, v6, 696.

13. Cox, H. C., Hardegger, E., Kögl, F., Liechti, P., Lohse, F., Salemink, C. A., *Helv. Chim. Acta*, 1958, v41, 229.

Received June 24, 1958. P.S.E.B.M., 1958, v98.

Alterations in Serum Proteins of Hibernating Hamsters.* (24217)

FRANK E. SOUTH, 2ND AND HENRY JEFFAY

Departments of Physiology and Biological Chemistry, University of Illinois College of Medicine, Chicago

In view of the manifold metabolic adjustments which accompany natural hibernation (1,2,7) it is not unreasonable for one to expect an alteration in the partitions of serum proteins during this state. That certain such changes may occur in at least the hibernating hedgehog (*Erinaceus europeueus*) was shown by Biörck *et al.*(3) whose data also indicated that there was no change in total serum protein level during hibernation in spite of a marked increase in hematocrit. The primary aim of our work was to determine what changes take place in serum proteins upon assumption of hibernation by the golden hamster (*Mesocricetus auratus*). We also have attempted to follow these changes in small series of cold exposed hamsters prior to onset of hibernal sleep and following spontaneous arousal therefrom.

Materials and methods. Other than control hamsters, maintained at room temperature, all animals were kept in a temperature controlled room at 5 ± 0.5°C. Experimental animals were divided into 3 groups: *Group 1* was exposed to the cold for various intervals of time, but had not hibernated; *Group 2* had hibernated continuously for 2 days; *Group 3* had been awake in the cold for one and 2 days after *spontaneous* arousal from hibernation. All animals were killed by cervical fracture. Blood for serum electrophoresis and hematocrit determinations was obtained directly from right ventricle after exposure of heart. Serum was analyzed for total protein by micro biuret method with pure bovine serum albumin serving as standard(8). Duplicate 0.1 ml samples of the serum were then subjected to paper electrophoresis using a Durrum type cell (Spinco commercial model R). After 20 hours at 5 ma. in a 0.05 M veronal buffer the electrophoretic strips were dried, stained with brom phenol blue and analyzed using the

* This work was supported by grant from Nat. Inst. Health, U.S.P.H.S. RG-5305.

TABLE I. Serum Proteins during Cold Exposure and Hibernation. Concentration expressed as g/100 ml.

		Total protein		Albumin		*Alpha*$_2$-globulin		*Alpha*$_1$-globulin		*Beta*-globulin		*Gamma*-globulin		Hematocrit	
Controls	(10)	7.30	.12*	2.68	.09	1.26	.11	1.02	.10	1.00	.11	1.36	.12	46.3	1.78
1 day cold exposed	(3)	6.13	.59	2.43	.09	.84	.08	.74	.18	1.43	.06	.711	.08	46.4	3.44
2 days " "	(3)	7.58	.46	2.45	.23	1.09	.05	1.42	.23	1.18	.16	1.115	.33	50.1	1.62
5 " " "	(3)	7.50	.73	2.90	.16	1.01	.06	.00	.10	1.55	.41	1.31	.32	51.8	1.89
10 " " "	(6)	7.42	.24	2.58	.20	1.33	.06	.82	.05	1.42	.26	1.17	.20		
2 mo " "	(5)	7.54	.12	2.43	.10	1.36	.07	6.81	.08	1.76	.09	1.20	.12		
Hibernating	(14)	8.87	.38	3.40	.07	1.38	.05	1.02	.08	1.74	.13	1.00	.10	62.2	.97
1 day after arousal	(5)	6.56	.33	2.39	.08	1.06	.05	.75	.07	1.14	.24	1.21	.24	50.9	.52
2 days " "	(3)	6.90	.11	2.19	.14	.876	.09	1.50	.22	1.49	.09	.911	.09	50.8	1.01

* Stand. error. No. of animals used are contained in parentheses.

Beckman Analytrol Scanner. Concentrations of the various protein fractions were calculated from the relative percentage of each fraction and the total serum protein content (Table I).

Results. The most obvious alterations in serum protein levels were related to the hemoconcentration that accompanied cold exposure and hibernation. Hematocrits of hibernating animals were about 34% higher than those of controls. Concentrations of total serum protein and serum albumin showed increases of 22 and 27% respectively ($P < 0.01$ in all cases. While albumin, the major serum protein, increased in concentration, the total circulating quantity of the fraction was probably not altered, since the rise was approximately equal to that of the hematocrit. Upon arousal, there occurred a general decrease in hematocrit and proteins approximating control or pre-hibernation levels. *Alpha*-globulins did not show a significant increase during hibernation but, considered on the basis of percentage of total protein, an actual decrease in total circulating amount was indicated. The reality of this decrease is questionable since it is well known that complete separation of these fractions from albumin by paper electrophoresis is usually poor, at best.

Of greater interest was the observation that the *beta*-globulins showed an increase prior to and during hibernation which was significantly greater than could be accounted for on the basis of simple concentration of serum proteins.

In contrast to the disproportionate increase in the *beta*-globulins was the marked decline which took place in *gamma*-globulin during torpidity.

Discussion. From data presented here, it would appear that changes in serum proteins of the hibernating hamster were brought about both by a simple hemoconcentration and through operation of other, as yet unexplained, factors. The hemoconcentration, as indicated by the more-or-less commensurate increases in hematocrit, total serum protein and serum albumin concentrations, confirms those data obtained by others studying other hibernating species(1,3,4). In partial conflict with our observations are those of Biörck *et al.*(3) on the hedgehog. These investigators found no change in total serum protein concentration during hibernation, in spite of the fact that the reported hematocrits rose significantly. Their results also differ from ours in that they found a decrease in *beta*-globulins whereas we found a significant increase.

Of the changes which occurred in serum proteins, and which could not be explained on the basis of hemoconcentration, the fall in *gamma*-globulin was the most striking. Whether this is due to a lack of stimuli for immunological reactions as suggested by Biörck(3) or to some metabolic peculiarity is not possible to ascertain at present.

Alterations in the circulating quantity of *alpha* and *beta* fractions raise the question of nutritive adaptations since *alpha*$_1$-globulin and *beta*$_1$-globulin are especially associated with blood lipids(5).

Upon examining the data, the possibility that the various fractions are differentially stored or removed from active circulation, or in the case of the *beta*-globulins released into

circulation from auxiliary stores, during hibernal sleep might be considered. In contrast to the hibernating hamster, the rabbit displays a decreased concentration of serum *beta*-globulins and an elevation of *gamma*-globulin during simple cold exposure(6).

Summary. 1) Alterations in various fractions of serum proteins and hematocrits were studied in hibernating and non-hibernating hamsters. 2) During hibernation the hematocrit, serum albumin and *beta*-globulins increased to a significant degree. The increase in *beta*-globulins was too large to be accounted for on the basis of simple hemoconcentration. Since concentrations of neither $alpha_1$ nor $alpha_2$-globulin increased, the data were interpreted to mean that total circulating quantity of these globulins decreased. A marked decline in concentration of *gamma*-globulin occurred.

The helpful cooperation of Dr. J. P. Marbarger in making available the facilities of the Aeromedical and Physical Environment Laboratory is gratefully acknowledged.

1. Lyman, C. P., Chatfield, P. O., *Physiol. Rev.*, 1955, v35, 403.
2. Kayser, C., *Ann. Biol.*, 1953, v29, 109.
3. Biörck, G., Johansson, B., Veige, S., *Acta Physiol. Scand.*, 1956, v37, 281.
4. Svihla, A., Bowman, H. C., *Am. J. Physiol.*, 1952, v171, 479.
5. Edsall, J. T., *Adv. Prot. Chem.*, 1947, v3, 458.
6. Waugh, D., *Am. J. Physiol.*, 1952, v168, 698.
7. South, F. E., Jr., *Physiol. Zool.*, 1958, v31, 6.
8. Schwartz, M., Myers, T. C., *Anal. Chem.*, 1958, v30, 1150.

Received June 24, 1958. P.S.E.B.M., 1958, v98.

Metabolism of Human Leukocytes *in vitro* V. Inhibition by Human Serum of Formate and Glycine Incorporation.*† (24218)

JOHN H. FRENSTER,‡ WILLIAM R. BEST AND RICHARD J. WINZLER
Departments of Medicine and Biological Chemistry, University of Illinois College of Medicine, Chicago

Human leukocytes can be isolated, incubated *in vitro*, and observed to incorporate labeled precursor compounds into cellular protein, nucleic acid, and acid-soluble components at rates characteristic of cell type and maturity(4). Incorporation of formate(4) and glycine(1) has been previously shown to be significantly more rapid in Krebs-Ringer-Bicarbonate-Glucose media than in normal human serum, suggesting that serum may contain inhibitors or dilutors of metabolic processes in which these precursors are involved. These findings may suggest the presence of a substance or substances in normal circulation which regulate metabolic processes in leukocytes. The physiological and clinical implications of such regulators might prove to be of great interest. Further studies of the effect of serum on formate and glycine incorporation into normal and leukemic leukocytes *in vitro*, therefore, were undertaken.

Materials and methods. Separation, incubation, and fractionation of leukocytes. Leukocytes were collected in citrate-fibrinogen, washed in saline, suspended in 1 ml of Krebs-Ringer-Bicarbonate-Glucose (KRBG)§ me-

* This work supported by Grants from Nat. Inst. of Health, P.H.S. Blood specimens were obtained from patients at Illinois Research and Educational, Cook Co., West Side Veterans Admin., and Presbyterian Hospitals, Chicago, and at Veterans Admin. Hospital, Hines, Ill. We wish to thank the many physicians from these institutions who aided us.

† The following preliminary abstracts have been presented: *Clinical Research Proc.*, 1957, v5, 147; Am. Hematologic Soc., Atlantic City, N. J., April 27, 1958.

‡ Present address: Rockefeller Institute for Medical Research, New York.

§ The following abbreviations have been used in this paper: KRBG = Krebs-Ringer-Bicarbonate supplemented with 3 mg/ml glucose; KRB = Krebs-Ringer-Bicarbonate; GPF = gross protein fraction; ASF = acid soluble fraction; CLL = chronic lymphocytic leukemia; CGL = chronic granulocytic leukemia; and AL = acute leukemia.