effect of cold on either hemoglobin or erythrocyte number was seen. Possibly 5 days are not a long enough time in which to expect acclimatization to appear, or possibly no adaptive change to low temperature occurs in hemoglobin or erythrocyte number. With acclimatization to cold some hemoconcentration results(8), which could account for the increase seen with the long cold exposure. The number of erythrocytes found in this study falls within the range listed for different strains of mice(20). Hemoglobin and blood sugar levels also agree with those reported for the same strain of mice(21).

Summary. Mice were exposed to cold (5°C) for periods of 15 minutes and 5 days and the effect on erythrocyte number, hemoglobin concentration and blood sugar level determined. The only significant change occurred in the blood sugar level during the short period of cold exposure. Statistically this change had a P value of $<.001$. Possible physiological changes are discussed.

1. Selye, H., *Stress*, Montreal, Canada, Acta, Inc., 1950.

2. Sellers, E. A., You, S. S., and Thomas, N., *Am. J. Physiol.*, 1951, v165, 481.

3. Leblond, C. P., and Gross, J., *Endocrinol.*, 1943, v33, 155.

4. Ershoff, B. H., and Golub, O. J., *Arch. Biochem.*, 1951, v30, 202.

5. Quimby, E. H., Werner, S. C., and Schmidt, C., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 537.

6. Gellhorn, E., and Feldman, J., *Am. J. Physiol.*, 1941, v133, 670.

7. Ciaccio, I., *Boll. Soc. Ital. Biol. Sperl.*, 1930, v5, 997.

8. Barbour, H. G., and Hamilton, W. F., *Am. J. Physiol.*, 1925, v73, 315.

9. Stein, H. J., Eliot, J. W., and Bader, R. A., *J. Appl. Physiol.*, 1949, v1, 575.

10. Freund, H., and Marchand, F., *Arch. Exp. Path. u. Pharm.*, 1913, v73, 276.

11. Kisch, B., Simons, A., and Weyl, P., *Biochim.*, 1929, v204, 179.

12. Geiger, E., *Magyar Orvasi. Archiv.*, 1926, v1.

13. Hallman, L. F., and Orent-Keiles, E., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 171.

14. Hiestand, W. A., and Miller, H. R., *Am. J. Physiol.*, 1944, v142, 310.

15. Van Middlesworth, L., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 476.

16. Feldman, J., Cortell, R., and Gellhorn, E., *Am. J. Physiol.*, 1940, v131, 281.

17. Stickney, J. C., Northup, D. W., and Van Liere, E. J., *ibid.*, 1951, v167, 559.

18. Sheard, C., and Sanford, A. H., *J. Lab. and Clin. Med.*, 1929, v14, 558.

19. Folin, O., and Wu, H., *J. Biol. Chem.*, 1928, v77, 421.

20. Snell, G. D., *Biology of the Laboratory Mouse*, Phila., Pa., Blakiston, 1941.

21. Hiestand, W. A., Hadley, M. F., Mercer, S. E., and Sandock, B. K., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 324.

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A Plasma Extract with Erythropoietic Activity. (21066)

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Speculation still exists regarding the precise mechanism by which anoxia, the so-called fundamental erythropoietic stimulus, evokes the formation of red cells in the higher organism. Mediation through a humoral factor, termed "hemopoietine", has been proposed as one type of explanation(1-3). Erythropoietic activity in sera obtained from rabbits rendered anemic by bleeding or exposed to lowered barometric pressures was not detected in another experiment(4). However, the largest quantities administered by these workers to intact or anemic rabbits did not exceed 15 ml daily. More recent experiments (5) have tended to renew interest in this problem because of the finding that larger quantities of sera (50 ml/day), obtained from bled rabbits, induced significant rises in the

numbers of peripheral red cells and reticulocytes, accompanied by increases in the percentages of nucleated erythrocytes within the bone marrow of intact rabbits.

Descriptions of the method of extraction and the properties of a protein-free fraction of plasma capable of increasing the rate of incorporation of labeled amino acids into proteins of rabbit reticulocytes have been published(6). The inference(7) that this factor and hemopoietine might be related prompted us to test the effects of this material upon the peripheral blood picture and bone marrow of the intact rat.

Materials and methods. Adult male rabbits were given daily subcutaneous injections, for a week, of one ml of a 2.5% solution of phenylhydrazine to induce intense anemia and reticulocytosis. At the end of the 7-day period, the red cell counts of the rabbits were reduced to approximately one mill/mm^3. Whole blood from these animals was collected in heparin (10 mg/50 ml blood). The plasma was brought to a pH of 5.5 by addition of 1 N HCl, boiled for 10 minutes and filtered (6). The filtrate representing 1/4 to 1/5 of the original volume of plasma was readjusted to a pH of 8.4 by addition of 1 N NaOH.* The material was placed immediately into 10 ml stoppered vials and stored in the freezing compartment of a refrigerator until used. Each of 6 normal adult female rats (150-200 g) was injected subcutaneously with 0.75 ml of this filtrate daily for 11 days. Six control rats received 0.75 ml of 1% saline solution for the same period of time. Estimations of peripheral blood values were made at intervals of 3 days for the entire experimental period and at 7-day intervals, for a period of 14 days, following cessation of treatment. Determinations were made of peripheral total red cell, total and differential white cell and total eosinophil numbers. Also included were estimations of reticulocyte percentages, hematocrit values, red cell sedimentation and fragility values, and bone marrow differentials, performed by previously described methods(8).

* All extracts were provided by the Princeton Laboratories, Inc., Princeton, N. J.

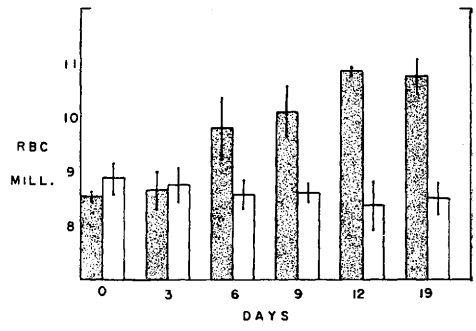

FIG. 1.

Results. Significant rises in red cell counts, hemoglobin concentrations, hematocrit values and reticulocyte percentages were induced by daily injections of the plasma extract†‡ (Figs. 1-4). The values tended to diminish after interruption of treatment. However, at 14 days after cessation of treatment (not shown in the Figs.) the red cell, hemoglobin and hematocrit values were still somewhat above the pre-injection levels.

No significant alterations were detected in the peripheral total, differential white cell and eosinophil numbers, or in the red cell sedi-

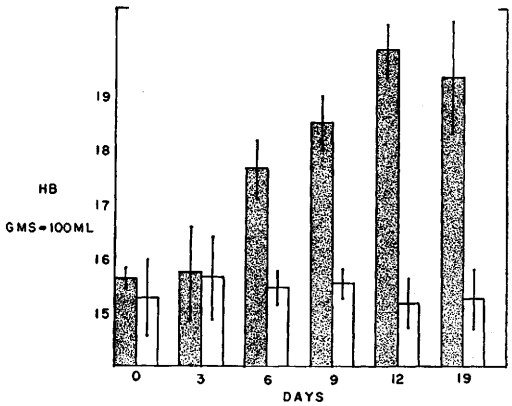

FIG. 2.

† Animals injected with the extracts exhibited no apparent toxic symptoms; their body weight gains throughout the experimental period were similar to those experienced by the saline-injected controls.

‡ The more potent plasma fractions were obtained from phenylhydrazine-treated rabbits displaying the most severe anemia and the greatest reticulocytosis (in some cases 90%). The results presented in this report are based on material obtained from such intensely stimulated donor rabbits.

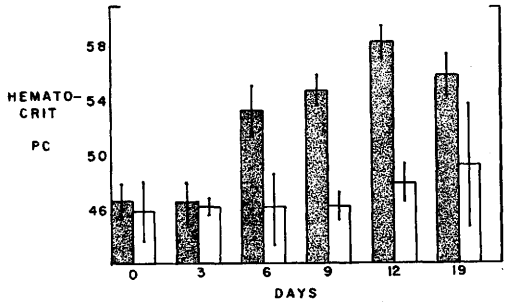

FIG. 3.

mentation and fragility values of the experimental and control animals.

The bone marrows of a group of intact rats, given 10 injections of the plasma extract, all appeared grossly reddened at the time of autopsy. Myelograms for these animals revealed approximately a 30% increase in the percentages of nucleated erythrocytes. None of the saline-injected controls exhibited these effects.

The experiments were repeated in 6 intact female rats with a lyophilized preparation of the plasma filtrate, prepared from phenylhydrazine-treated rabbits. It was reconstituted with distilled water just prior to administration. The effects of this material upon the peripheral erythrocytic values were similar to, but not as marked, as those obtained in the first series of experiments. Boiled plasma extracts obtained from untreated intact rabbits were found to be without influence upon the various red cell values

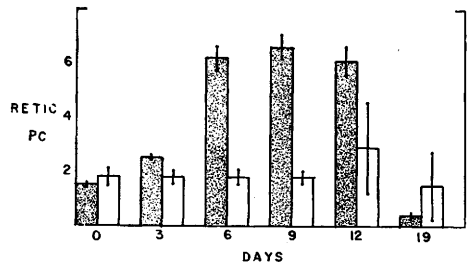

FIG. 4.

Fig. 1-4 represent alterations in red cell counts, hemoglobin concentrations, hematocrit values and reticulocyte percentages in intact rats injected daily for 11 days with 0.75 ml of an extract obtained from the plasma of phenylhydrazine-treated rabbits (stippled bars) and controls injected for same time with 0.75 ml of 1% saline solution (empty bars). Mean values are indicated. The vertical lines at the tops of the bars represent ± 1 stand. error of the mean.

in a group of 5 female rats tested.

Discussion. The results point clearly to the existence of a factor (or factors) in the plasma of phenylhydrazine-treated rabbits with strong erythropoietic properties when tested in the intact rat. It seems unlikely that the alterations in peripheral erythrocytic values are due to hemoconcentration effects in view of the accompanying reticulocytosis, the increases in the percentages of nucleated erythrocytes within the marrow and the absence of significant alterations in the numbers and proportions of the peripheral leucocytic elements.

Erythropoietic activity in a boiled acid extract of urine obtained from repeatedly bled rabbits has been reported(9). Inability to observe activity in similarly prepared plasma from these animals may have been due to the use of only one injection of this material. Unpublished studies in our laboratories have shown that boiled filtrates of plasma secured from rabbits subjected to repeated bleedings over a period of 3 weeks, and showing average red cell counts of 2 million/cmm and reticulocyte values of 20% at the time of sacrifice, also possess erythropoietic activity when administered daily over a 10-day period. These filtrates, however, were not as potent as those secured from the more intensely anemic phenylhydrazine-treated rabbits.

Studies are now in progress to obtain a more detailed knowledge of the chemical nature of the factor and the mechanism of its action in the intact and anemic animal. Use of this factor in a purified form could be made conceivably in patients subject to various slowly-responding or refractory anemias.

Summary. Administration to intact rats of an extract, prepared from the plasma of phenylhydrazine-treated rabbits, results in significant increases in peripheral red cell counts, hemoglobin concentrations, reticulocyte percentages, hematocrit values, and in the concentrations of nucleated erythrocytes within the marrow. The extract exerts no significant influence upon the total and differential white cell numbers or the sedimentation rates and red cell fragility values. The factor may be related to, or identical with, the cir-

culating "hemopoietine", hypothesized to be the humoral mediator of anoxia, the fundamental erythrocytogenic stimulus.

1. Carnot, P., and Deflandre, C., *Compt. rend. Acad. sc.*, 1906, v143, 384.

2. Gibelli, C., *Arch. f. exp. Path. U. Pharmakol.*, 1911, v65, 248.

3. Forster, J., and Kiss, F., *Biochem. Z.*, 1925, v160, 442.

4. Gordon, A. S., and Dubin, M., *Am. J. Physiol.*, 1934, v107, 704.

5. Erslev, A., *Blood*, 1953, v8, 349.

6. Borsook, H., Deasy, C. L., Haagen-Smit, A. J., Keighley, G., and Lowy, P. H., *J. Biol. Chem.*, 1952, v196, 669.

7. Grant, W. C., and Root, W. S., *Physiol. Rev.*, 1952, v32, 449.

8. Gordon, A. S., Piliero, S. J., and Landau, D., *Endocrinology*, 1951, v49, 497.

9. Hodgson, G., and Tohá, J., *Blood*, 1954, v9, 299.

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Sensitivity Tests to Insulin in Patients with Local Skin Lesions from Insulin. (21067)

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(Introduced by J. H. Sandground.)

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A considerable number of diabetic individuals experience local skin reactions following the administration of insulin in the conventional manner. In previous years these were ascribed to chemical irritation arising either from the alcohol used for sterilization of syringes and needles, from the preservatives added in the manufacture of insulin or from the acidity of the earlier insulin preparations. At present, most workers consider these local manifestations as hypersensitivity to either the impurities within the insulin preparations, to the insulin protein itself or to the pancreatic protein of the species from which the hormone is derived. Recently Fabrykant and Ashe (10,10a) reported that skin changes at the site of insulin injections could be prevented by introducing the hormone into the deeper layers of the subcutaneous tissue.

The present investigation is concerned with sensitivity studies in a group of patients presenting local skin reactions while on insulin therapy in order to shed more light on the genesis of such manifestations.

Plan of study. Sixteen diabetic patients, whose local dermal reactions to insulin therapy consisted of nodules with or without erythema or stinging, were tested intradermally on the outer aspects of the arms with 0.02 cc of several insulin products in a variety of dilutions, as well as with commercial extract of beef and pork. Control tests were performed with the same quantity (0.02 cc) of Coca's diluting fluid. A group of 16 non-diabetic controls who were free from any skin affection, were similarly tested with the same materials and technic. The results were read 2, 5 and 10 minutes after the injection and were interpreted as negative, slight, moderate, marked or any combination of the last three, depending on the size of the wheal, the degree of erythema and the presence of pseudopods. For example, if the injected material did not produce a wheal greater than that from the control solution, the reaction was considered negative; if the wheal was only a little larger and a small area of erythema had developed, the reaction was termed slight; and if the wheal was more than twice the size produced by the original amount administered, with a considerable areola and the appearance of pseudopods, it was designated marked. A moderate reaction was one between these two. Some patients presented a transition from one type of reaction to another, and this was interpreted as slight-to-moderate or moderate-to-marked. We have considered as significant only the slight-to-moderate, moderate and