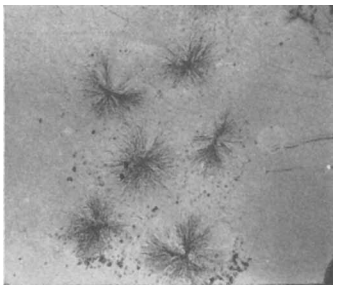

FIG. 1. Microphotograph of osazone crystals obtained from the virulent plague strain, 337/L.

tein of plague bacilli. The same method as used in the case of bacterial debris was employed for preparing osazone crystals from the specific plague proteins, L auto P1/3 and L_5P1/3. The same type of crystals in a purer form was obtained in both of them, the melting point being 166°-168°C.

Comments. In the attempt to isolate polysaccharide from the supernatant of the Haffkine plague vaccine prepared in the enriched casein hydrolysate broth a very small quantity (23.5 mg) could be isolated from 7 liters of the vaccine. From the nature of its chemical and serological reactions it seems that the substance is intimately associated with the specific soluble proteins for the protective plague strains. This has been confirmed by isolating an arabinose-like osazone (melting point 166°-168°C) from the plague proteins, L_5P1/3 and Lauto P1/3. The same osazone crystals were found in the protected plague strains 139/L and 53/Hav but not in the avirulent non-protective plague strains TRU or in the pseudotuberculosis strains, PR/I and PR/IV. These findings lend support to the view that the specific protective substance of plague bacilli is a polysaccharide-protein complex, probably a nucleoprotein.

Summary. A polysaccharide yielding osazone resembling that of *Arabinose* with a melting point of 166°-168°C has been isolated from the supernatant of the Haffkine plague vaccine as well as from the specific soluble protein and, to a less extent, from the bacterial debris of both virulent and avirulent protective plague strains. It is absent in non-protective avirulent plague and pseudotuberculosis organisms. The protective substance of the plague bacillus is probably a polysaccharide-protein complex.

This work was carried out under the active support of Major-General Sir Sahib Singh Sokhey, the late Director, Haffkine Institute, Bombay.

Received May 11, 1951. P.S.E.B.M., 1951, v77.

Serum Iron and Hemoglobin Values of 275 Healthy Women.* (18890)

MARILYN CHALOUPKA, RUTH M. LEVERTON, AND ETHEL DIEDRICHSEN.
(Introduced by C. E. Georgi.)

From the Human Nutritional Laboratory, Nebraska Agricultural Experiment Station, Lincoln, Nebr.

The importance of serum iron in the intermediary metabolism of iron is recognized. Many investigators have reported serum iron values for women and these values range from a low of 30 μg % as reported by Eckerström (1) to a high of 210 μg % as reported by Vahlquist(2). There is some variation in the range of values which is considered by different investigators to be normal for women. Dahl(3), who has studied the greatest number of subjects recently, suggests 70 μg to 140

* Contribution No. 3, Subproject—"The Nutritional Status and Dietary Needs of Older People" of the North Central Cooperative Project NC-5 "Nutritional Status and Dietary Needs of Population Groups." In cooperation with the Bureau of Human Nutrition and Home Economics, U. S. Department of Agriculture. Published with the approval of the Director as paper no. 537 Journal Series, Nebraska Agric. Exp. Station.

1. Eckerström, S., *Acta Med. Scand.*, 1944, v118, 69.

2. Vahlquist, B. C., *Acta Paediat.*, 28, suppl. 5, 1941.

3. Dahl, S., *Brit. Med. J.*, 1948, v1, 731.

μg % as the normal range of serum iron values for women; Moore *et al.*(4) suggest the wider range of 50 μg to 180 μg %; and, although Vahlquist(2) considers 50 μg % to be the lower limit of normality, he states that the upper limit must be placed considerably above 200 μg %. Some investigators have found that menstruation affects the serum iron level. Dahl(3) states that serum iron values were lower for women before the menopause than after. Within a single menstrual cycle he found the highest values shortly before menstruation and the lowest during and immediately after. Powell(5) confirmed this trend but Cartwright *et al.*(6) and Brøchner-Mortensen and Olsen(7) found no relationship between serum iron values and different times during a menstrual cycle. Age is another factor which has been found to affect serum iron levels in some cases. Eckerström (1) compared mean serum iron values of 100 men and women, 70 to 94 years old, with values of adults studied by Vahlquist(2) and found the elderly subjects had lower serum iron values. Brøchner-Mortensen and Olsen (7), however, reported no dependency of serum iron levels upon age. Cartwright *et al.* (6) agreed with this finding although only one-third of their 43 subjects were older than 30 years.

In a study of the nutritional status of healthy women in this laboratory it seemed pertinent to include an evaluation of serum iron levels.

Procedure. Serum iron and hemoglobin values were determined for 275 healthy women who were between the ages of 17 and 86 years and were eating self-chosen diets. The 17- to 25-year-old women were college students, the 30- to 86-year-old women were homemakers and business and professional women. About half of the subjects had children, the number ranging from one to 7. With few exceptions all subjects were above average in socio-economic status. Fasting blood samples, obtained in the early morning by venipuncture, were used for the determination of serum iron and hemoglobin. Samples were not taken during the first 3 days of menstruation. A protein-free extract was prepared by precipitation and heating of the serum as described by Kitzes *et al.*(8). Iron was determined in this extract by the method of Pohle *et al.*(9). The optical density of the colored complex formed by 1, 10-phenanthroline and the reduced iron was measured in a Beckman spectrophotometer for which the extinction coefficient of iron had been determined. Precautions were taken to avoid hemolysis of the whole blood. Glass-distilled water and iron-free reagents were used. Hemoglobin was converted to oxyhemoglobin according to the method of Todd and Sanford (10) using 0.1% Na_2CO_3 and the depth of color determined in a Sheard-Sanford photelometer which had been calibrated with blood of a known oxygen capacity.

Results. Serum iron and hemoglobin values were sorted into groups according to the ages of the women. The mean values and standard deviations for serum iron and hemoglobin are given in Table I for each of the 7 age groups and for the combined groups.

Serum iron values ranged from 33 μg to 221 μg % and the mean value for all subjects was 116 ± 33 μg %. The youngest group, 17 to 25 years, had a mean serum iron value of 125 μg %. With the exception of 122 μg % for the 50 to 59 year group the mean values for each successive age group declined to 95 μg % for the 70 to 79 year group, then rose slightly to 101 μg % for the oldest group, 80 to 86 years. Analysis of variance indicated that a significant difference in serum iron values existed among the different age

4. Moore, C. V., Arrowsmith, W. R., Quiligan, J. J., Jr., and Read, J. T., *J. Clin. Invest.*, 1937, v16, 613.

5. Powell, J. F., *Quart. J. Med.*, 1944, v13, 19.

6. Cartwright, G. E., Huguley, C. M., Jr., Ashenbrucker, H., Fay, J., and Wintrobe, M. M., *Blood*, 1948, v3, 501.

7. Brøchner-Mortensen, K., and Olsen, C., *Comptes Rendus des Travaux du Lab.*, Carlsberg, Serie Chim., 1940, v25, 235.

8. Kitzes, G., Elvehjem, C. A., and Schuette, H. A., *J. Biol. Chem.*, 1944, v155, 653.

9. Pohle, W. D., Cook, J. H., and Mehlenbacher, V. C., *Food Research*, 1947, v12, 229.

10. Todd, J. C. and Sanford, A. H., *Clinical Diagnosis by Laboratory Methods*, 8th Ed. p242, Philadelphia, W. B. Saunders Co., 1937.

TABLE I. Serum Iron and Hemoglobin Values of Women in This Study.

Age groups	No. of subjects	Mean age, yr	Serum iron		Hemoglobin	
			Mean value	Std dev	Mean value	Std dev
			μg %		g %	
17-25	52	20	125	31	13.2	.8
30-39	35	34	119	40	13.2	.9
40-49	63	44	113	35	13	.9
50-59	64	55	122	30	13	.8
60-69	32	63	110	34	13.4	.9
70-79	22	74	95	21	13.4	.8
80-86	7	83	101	17	13.5	1.2
All	275		116	33	13.2	.9
Range		17-86	33-221		10.4-16.5	

TABLE II. Serum Iron and Hemoglobin Values Related to Menopause.

Stage	No. of subjects	Serum iron		Hemoglobin	
		Mean value	Std dev	Mean value	Std dev
		μg %		g %	
Pre-menopause	30	114	37	13	.8
During "	23	105	36	13.2	.9
Post "	18	115	22	12.9	.7

groups ($F = 3.19$; $F_{.01} = 2.90$). The regression coefficient of serum iron on age was —.343. The t test showed this coefficient to be significant at the 1% level of probability ($t = 3.014$; $t_{.01} = 2.592$).

The mean hemoglobin value for all subjects was 13.2 ± 0.9 g % with a range of 10.4 g to 16.5 g %. No significant difference among the mean hemoglobin values existed for the different age groups nor was there any correlation between the hemoglobin values and the serum iron. Nineteen of the women had hemoglobin values below 12.0 g %, or from 10.4 to 11.9 g %. These low values indicated anemia, although medical records did not show any other symptoms of abnormality. The mean serum iron and hemoglobin values for the 19 women were 107 μg % and 11.6 g %, respectively. When the serum iron values of these women were omitted from the entire group, the mean value of the remaining 256 women was 117 ± 33 μg %, as compared with 116 ± 33 μg % for the 275 women. The range of serum iron values and of ages remained unchanged. Omission of the hemoglobin values of these 19 women from the group raised the mean hemoglobin value from 13.2 ± 0.9 g % to 13.3 ± 0.8 g %.

The relationship of serum iron values to menopause was investigated. The women who were going through the menopause ranged in age from 40 to 52 years. Therefore, the serum iron and hemoglobin values for all women in this age range were grouped on the basis of their relation to the menopause; that is, pre-menopause, during menopause, or post-menopause, and the results are presented in Table II. The mean serum iron values were 114 ± 37, 105 ± 36, and 115 ± 22 μg %, respectively. An analysis of variance indicated that no significant difference existed among these values.

Discussion. Serum iron values of 33 μg to 221 μg % found in this study constitute a wider range than has been considered normal by many investigators. Five per cent of the women had values below 68 μg % and 5% had values above 180 μg %. Careful scrutiny was given to the records of the thorough medical and physical examinations of these women and no abnormalities were observed which could account for either the low or the high values for serum iron. In only one case was a low serum iron value, 58 μg %, accompanied by a low hemoglobin value, 10.7 g %.

In view of the many physiological factors which normally influence the level of serum iron in the blood a wide range of normal values is not surprising. These factors include the rate of iron absorption, the balance between iron entering and leaving the tissues, the size of storage depots, and the rate of the

production and breakdown of hemoglobin. Abnormal serum iron values may be caused by severe blood losses and certain pathological conditions.

No explanation is apparent for the increased mean serum iron value of 122 μg % for the 50 to 59 year group as compared with 113 μg % in the preceding and 110 μg % in the following decade. The mean value for the women in the first half of this decade was the same as that for women in the last half. Therefore this higher value cannot be related to age within the decade.

Approximately one-third of the women between the ages of 40 and 52 years were going through the menopause. Their mean serum iron value was 9 μg % lower than women in this age range who had not reached the menopause, and 10 μg % lower than those who had passed the menopause. The difference, however, was not significant.

The nutritional and medical significance of the decrease of serum iron with advancing age in healthy women is not apparent. The process of aging may modify those physiological factors which influence the level of serum iron in such a way as to cause this decrease.

Summary. (1) The serum iron and hemoglobin values were determined for 275 healthy women ranging in age from 17 to 86 years. Mean values for all women were 116 ± 33 μg % and 13.2 ± 0.9 g %, respectively. (2) Analysis of variance indicated a difference of serum iron levels among different age groups which was significant at the 1% level. The regression coefficient of serum iron on age was —.343, indicating a decrease of serum iron with advancing age. This decrease was not accompanied by a decrease in hemoglobin values, nor was there a correlation between serum iron and whether the menopause had been reached or passed. (3) There is no apparent nutritional or medical significance to the decrease of serum iron levels with age in healthy women.

Received May 18, 1951. P.S.E.B.M., 1951, v77.

Pressure Pulse Recording from an Isolated Mammalian Heart Preparation Based on a New Principle.* (18891)

DAVID F. OPDYKE.†

From the Department of Physiology, Western Reserve University School of Medicine, Cleveland, Ohio.

Certain problems of cardiodynamics can be studied profitably by the use of an improved isolated mammalian heart preparation. The mode of relaxation of the ventricle, for example, may hold further information concerning the control of cardiac output, for it is increasingly apparent that the remarkable property of the heart is not only that it can pump so much, but also that it can fill so efficiently. A study of the relaxation process of the ventricle is difficult, however, when large volumes of blood are being pumped by the heart.

In order to study the relaxation process adequately an isolated heart preparation should possess the following attributes: (a) generate ventricular pressure pulses of normal contour, (b) possess easy control of atrial and ventricular systolic and diastolic pressures, (c) pump no blood, *i.e.*, have a fixed ventricular volume, and (d) not deteriorate over a period of many hours. The preparation to be described possesses all these characteristics to a satisfactory extent.

The preparation. Heyman and Kochman (1) apparently introduced the idea of using a donor dog to supply the blood and pressure head for the perfusion of the coronary arteries of the isolated heart. Although their

* Supported by a grant from the Life Insurance Medical Research Fund.

† Present address, Merck Institute for Therapeutic Research, Rahway, N. J.

1. Heyman, J. F., and Kochman, M., *Arch. Pharm. et de Therap.*, 1904, v13, 379.