and causes development of coronary atherosclerosis or is coincident with it and not an etiologic agent has not been clarified. This is being investigated.

Summary. Renal hypertensive rats on an all-meat diet developed hypercholesterolemia and coronary atherosclerosis within 16 weeks. Intact meat-fed, intact chow-fed, and renal hypertensive chow-fed rats developed neither condition by 50, 20 and 20 weeks respectively. Aortas of all animals chow- or meat-fed were normal. Thus, it appears that hypertension coupled with a high fat, high protein, but low cholesterol, diet is related to the production of hypercholesterolemia and coronary atherosclerosis. It is suggested that such a system may serve as an *in vivo* tool for the study of prevention, development and possible reversal of coronary atherosclerosis.

1. Sabiston, D. C., Jr., Smith, G. W., Talbert, J. L., Gutelius, J., Vasko, J. S., *Ann. Surg.*, 1961, v153, 13.

2. Grollman, A., PROC. SOC. EXP. BIOL. AND MED., 1944, v57, 102.

3. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., *J. Biol. Chem.*, 1944, v153, 375.

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Further Evidence for Reabsorption of Hemoglobin Iron Lost into the Intestine in Hookworm Infected Subjects.* (27425)

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Roche *et al.*(1,2) Foy and Kondi(3) Tasker(4) and Layrisse *et al.*(5) showed that considerable blood may be lost into the intestine by patients infected with hookworm. By means of a method involving the tagging of circulating erythrocytes with both Fe 59 and Cr 51(6), Roche and Pérez-Giménez(7) and Layrisse *et al.*(5) were able to measure intestinal iron loss as distinguished from fecal iron loss. It was found that fecal loss was on an average 55.9(7) and 64.10%(5) of intestinal loss, which would indicate that part of the hemoglobin iron was being reabsorbed. There was excellent correlation between fecal Fe 59 loss and reduction in blood Fe 59 activity, which would indicate that the absorbed iron was being utilized for fresh red cell synthesis (5).

Barun *et al.*(8) demonstrated, in hematologically normal patients injected with Fe 59, a progressive migration of radioactivity from the top to the lower levels of the packed red cell mass, as the erythrocytes aged with time. Eventually, radioactivity would again increase in the upper layer as the Fe 59 from the destroyed red cells was utilized for a new erythrocyte formation. Somewhat more efficient separation of erythrocytes of different age has been obtained by ultracentrifugation (9).

It was thought that, in cases with an abnormal iron loss into the intestine, as in hookworm infected subjects, followed by reabsorption and reutilization of such iron for hemoglobin synthesis, a constant supply of Fe 59 into freshly formed erythrocytes should result in radioactivity in the top layer of the hematocrit remaining at a relatively high level. If proved unequivocally to be true, this finding would provide further proof for iron reabsorption and reutilization from the intestinal tract.

Methods. Four heavily infected patients, with more than 5000 hookworm ova per gram of feces, and 3 normal non-infected subjects were selected for study. The patients were hospitalized in the wards of the Hospital Universitario in Caracas throughout the study

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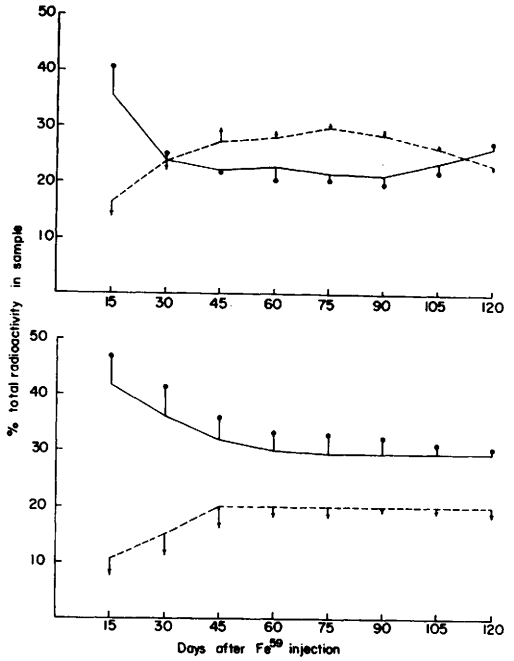

FIG. 1. Distribution of Fe 59 activity in upper and bottom layer of packed red cells. Above: avg of 3 normal subjects. Below: avg of 4 patients heavily infected with hookworm. Full line: radioactivity of upper layer. Interrupted line: radioactivity of bottom layer. Vertical lines ending with a dot: upper layer stand. dev. Vertical lines ending with an arrow: bottom layer stand. dev.

and no iron therapy was given. Circulating erythrocytes were tagged by injecting 15 μc of Fe 59, with a specific activity of about 40 μc/mg of iron in the form of ferrous citrate. Twenty cc of blood was withdrawn every 15 days for 120 days. Blood samples were prepared by means of a modification of the original method of Barun *et al.*(8). The blood was collected in heparinized tubes; 2 cc was used for hematocrit, red cell count and hemoglobin determinations, and the rest was left standing about one-half hour. The plasma was separated off and the remaining red blood cells were centrifuged at 3000 rpm (2260 *g*) for 45 minutes. The packed red cells were then marked off into 4 equal parts, and each portion was drawn off separately by means of a steel tube attached to a 2 cc syringe. The syringe and needle were washed twice between each portion, and the washing added to the previous sample. The samples were frozen and thawed until complete hemolysis ensued and radioactivity was counted in a well type scintillation counter. The result was expressed in percent of total radioactivity found in the 4 layers.

Results. The hemoglobin and hematocrit values in all subjects were constant throughout the study, indicating that iron metabolism was in equilibrium. The results are summarized in Fig. 1. Only values from the top and the bottom layer were utilized, following the practice of Barun *et al.*(8) as those in the middle layers turned out to be too variable to give readily interpretable results. Although the subjects were few, the results were uniform and the standard deviations small, particularly after the 15-day sample. The curves in the normal and in the hookworm infected subjects are fundamentally different; in the former, the lines cross around the 30th day, radioactivity in the lower layer becoming higher than that in the upper. Around the 80th day, radioactivity in the upper layer starts increasing, and that in the lower layer decreasing, until the curves cross again around the 110th day. In hookworm-infected subjects, although there is an initial increase and decrease respectively of the upper and lower level radioactivity, the values remain separated and the curves never cross.

Discussion. The results in normal subjects are in essential agreement with those given by Barun *et al.*(8). Since incorporation of Fe 59 is limited to immature erythrocyte series(10,11), and the tagged cells which appear in the circulation during the first days after Fe 59 administration are mainly reticulocytes whose density is relatively low(12,13), radioactivity in the top layer of the packed cells would be expected to be initially high, which is the case. As the cells grow older, their density increases(14,15) and Fe 59 radioactivity migrates towards the bottom of the tube, which accounts for the reduction in activity in upper hematocrit levels, and the progressive increase in lower level activity. As the older tagged cells begin to be destroyed, around the 90th day(14,15), radioactivity in the bottom layer decreases and, as the iron from these destroyed red cells is reutilized for erythrocyte synthesis, radioactivity in the upper layer again increases.

In the hookworm-infected subject, the up-

per level radioactivity remains elevated. This is interpreted as due to continuous incorporation of young Fe 59 labelled erythrocytes into this layer. It is not due to increased hemolysis, because, if such were the case, one would expect a decrease of bottom layer radioactivity before the 90th day or graphic evidence for occurrence of a homogeneous pool between the 2 layers(8), as may occur in patients with very short erythrocyte life span (unpublished experiment). In hookworm-infected patients, practically the only source of Fe 59 for hemoglobin synthesis in the marrow is the Fe 59 reabsorbed from the hemoglobin which is constantly being lost into the intestine. As a consequence of this loss, the patients may destroy 5 times as many erythrocytes as normal and but few of the newly released red cells reach old age; under such circumstances, at the 30th day only 23% of the cells would remain in the circulation and at 80th day, less than 2%. This accounts for the bottom layer of the hematocrit remaining constantly low. By the same token, this finding provides indirect evidence for reabsorption and reutilization of Fe 59 from hemoglobin lost into the gastrointestinal tract by hookworm-infected patients.

Summary. The distribution of Fe 59 in the top and bottom layer of centrifuged erythrocyte samples was determined during 120 days after administration of the isotope in hematologically normal subjects and hookworm-infected patients. While in normal subjects there was a progressive migration of radioactivity from the top to the bottom layer between the 15th and 90th days as the erythrocyte aged with time, in the hookworm-infected patient the top radioactivity layer remained constantly high during the 120 days of the study. This phenomenon is explained by the constant reabsorption of iron from the hemoglobin lost into the intestine, which is reutilized by the bone marrow to form new erythrocytes.

1. Roche, M., Pérez-Giménez, M. E., Layrisse, M., Di Prisco, E., *J. Clin. Invest.,* 1957, v36, 1183.

2. Roche, M., Pérez-Giménez, M. E., Layrisse, M., *Am. J. Digest. Dis.,* 1957, v2, 265.

3. Foy, H., Kondi, A., *Tr. Roy. Soc. Trop. Med. Hyg.,* 1960, v54, 419.

4. Tasker, P. W. G., *ibid.,* 1961, v55, 36.

5. Layrisse, M., Paz, A., Blumenfeld, N., Roche, M., *Blood,* 1961, v18, 61.

6. Roche, M., Pérez-Giménez, M. E., Levy, A., *Nature,* 1957, v180, 1278.

7. Roche, M., Pérez-Giménez, M. E., *J. Lab. and Clin. Med.,* 1959, v54, 49.

8. Barun, E. R., Figueroa, W. G., Perry, S. M., *J. Clin. Invest.,* 1957, v36, 676.

9. Rigas, D. A., Koller, R. D., *J. Lab. Clin. Med.,* 1961, v58, 242.

10. Walsh, R. J., Thomas, E. D., Chow, S. K., Fluharty, R. G., Finch, C. A., *Science,* 1949, v110, 396.

11. Lajtha, L. G., Suit, H. D., *Brit. J. Haemat.,* 1955, v1, 55.

12. Finch, C. A., Wolf, J. A., Rath, C. E., Fluharty, R. G., *J. Lab. and Clin. Med.,* 1949, v34, 1480.

13. Dreyfus, J. C., Schapira, G., Druh, J., *Compt. rend. soc. de biol.,* 1950, v144, 792.

14. London, I. M., Shemin, D., West, R., Rittemberg, D., *J. Biol. Chem.,* 1949, v179, 463.

15. Eadie, G. S., Brown, I. W., Jr., *Blood,* 1953, v8, 1110.

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