The substitution of 20% lard for an equivalent amount of corn increased the caloric density of the diet by approximately 40% and the ratio of calories to dietary protein by about 50%. The carbohydrate content of the diet was similarly reduced. These altered relationships may have been involved in the increased requirement for vit. B_{12} observed with the high fat diet.

Further discussion of the mechanism by which the high fat diet increased the chick's vit. B_{12} requirement will be considered more appropriately in a subsequent report on the "sparing" effect of certain nutrients upon vit. B_{12} under these conditions.

The graded growth response to varying levels of vit. B_{12} which has been obtained, makes diet C30 suitable for assessing the vit. B_{12} activity of concentrates or analogues for the chick. This diet has proven very useful in our laboratory for this purpose(11). The addition of other nutrients, such as methionine or choline, may alter the chick's response to vit. B_{12}, therefore, this diet cannot be used to evaluate the vit. B_{12} activity of low potency materials.

Summary. Incorporation of 20% fat into a corn-soybean oil meal diet increased ten to twenty-fold the vit. B_{12} requirement of non-depleted chicks for optimal growth at 4 weeks of age. The requirement was between 0 and 5 γ per kg of diet (3% fat) and between 50 and 100 γ with the high fat diet. The high dietary fat did not deplete the chick of its vit. B_{12} liver store or alter the absorption of dietary vit. B_{12} and its subsequent storage in the liver.

The authors gratefully acknowledge the technical assistance of Clarence E. Emery, Helen M. Hood, and Woodrow W. Duvall.

1. Ott, W. H., Rickes, E. L., and Wood, T. R., *J. Biol. Chem.*, 1948, v174, 1047.

2. Lillie, R. J., Denton, C. A., and Bird, H. R., *ibid.*, 1948, v176, 1477.

3. Stokstad, E. L. R., Jukes, T. H., Pierce, J., Page, A. C., Jr., and Franklin, A. L., *ibid.*, 1949, v180, 647.

4. Ott, W. H., *Poultry Sci.*, 1951, v30, 86.

5. Davis, R. L., and Briggs, G. M., *ibid.*, 1951, v30, 628.

6. Miller, R. F., Norris, L. C., and Heuser, G. F., *ibid.*, 1956, v35, 342.

7. Committee on Animal Nutrition, National Research Council, Pub. 301, 1954, 6.

8. Milligan, J. L., Arscott, G. H., and Combs, G. F., *Poultry Sci.*, 1952, v31, 830.

9. Smith, E. L., *Nutrition Abst. and Rev.*, 1951, v20, 795.

10. Spivey, M. R., Briggs, G. M., and Ortiz, L. O., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 451.

11. Briggs, G. M., and Fox, M. R. Spivey, *ibid.*, 1955, v89, 318.

12. Briggs, G. M., Spivey, M. R., Keresztesy, J. C., and Silverman, M., *ibid.*, 1952, v81, 113.

13. *The Pharmacopeia of the United States of America, Fifteenth Rev.*, 1955, 885.

Received September 12, 1956. P.S.E.B.M., 1956, v93.

Effects of Hypoxia on Iron Absorption in Rats. (22798)

PHILIP S. VASSAR* AND DAVID M. TAYLOR (Introduced by D. H. Copp)
Radiobiological Research Unit, Institute of Cancer Research, Royal Cancer Hospital, London, England.

Many studies have shown that gastro-intestinal absorption of iron is increased, in experimental animals(1,2,3) and human subjects(4), during many diverse types of anemia. Granick(5) has suggested that accompanying hypoxia is probably the only common factor capable of increasing iron absorption.

The present experiments were designed to test the hypothesis that hypoxia is capable of increasing iron absorption without the complicating presence of anemia or dietary changes.

* Present address: Department of Pathology, Faculty of Medicine, University of British Columbia, at Vancouver General Hospital, B.C.

Methods and materials. We used young male rats of the August strain, each weighing about 120 g. The animals were fed exclusively on M.R.C. diet No. 41,† food and water being freely allowed. During experiments the animals were housed singly in metabolism cages. For the hypoxia experiments the cages were placed in an airtight glass tank through which a gas mixture, consisting of 15% oxygen and 85% nitrogen, was passed at 3 liters per minute. In the corresponding control experiment air was passed through the tank at the same rate. Radioactive iron (Fe^{59}) was administered by stomach tube while the animal was lightly anaesthetized with ether. The amount of iron administered to each rat was 0.6 μc $Fe^{59}Cl_3$ (less than 1 μg Fe) contained in 0.2 ml acetate buffer at pH 5. The following experiments were performed: 1. Controls: 8 animals were exposed to air at 3 liters flow/minute for 48 hours, then given Fe^{59} and immediately replaced in the same environment for a further 24 hours. 2. 9 animals were exposed to the hypoxic atmosphere for 48 hours, then given Fe^{59} and immediately replaced in the hypoxic atmosphere for a further 24 hours. In the remaining group, animals were kept at normal room atmosphere. 3. 27 control animals were given only Fe^{59}. In each case, feces were collected daily for 6 days. Feces were placed in small glass tubes and their radioactivity assayed by direct counting in a well-type scintillation counter (efficiency for Fe^{59} approximately 10%). Fe^{59} was administered at approximately the same time of day in each experiment, and it was noted that food intake and volume of fecal excretion were normal during experiments.

Results. The results of the 3 experiments are summarized in Table I. There was no significant difference between iron absorption in free air and in the glass tank at 3 liters/minute. However, in Exp. 2, where animals were exposed to 48 hours of hypoxia prior to Fe^{59} administration, there was a significant increase in iron absorption by average of 31%. ($P<0.001$).

† Medical Research Council diet No. 41 is a balanced diet with adequate mineral content.

TABLE I. Fe^{59} Absorption in Control and Hypoxic Rats.

	No. of animals	Mean absorption, % of administered dose	Range, %
1. Control: air, 3 l/min.	8	24 ± 2.83*	16-40
2. Hypoxia (15% O_2), 3 l/min.	9	55 ± 5.0	37-76
3. Control: free air	27	33 ± 1.54	16-47

* Stand. error of mean.

Throughout these experiments it was found that Fe^{59} excretion was maximal throughout the first 24 hours and thereafter fell off rapidly to negligible amounts after 6 days.

Discussion. These results confirm the hypothesis that hypoxia is capable of increasing absorption of iron from the gastro-intestinal tract without the presence of anemia or dietary changes.

The mechanism by which hypoxia increases gastro-intestinal absorption of iron remains obscure. However, recently it has been shown that hypoxia causes a redistribution of tissue iron(6) and an increase in chromoprotein oxygen transport systems(7,8). Possibly iron is mobilized from the intestinal mucosa for this purpose, under the influence of hypoxic stress; however, these processes probably occur too slowly to account for our results. On the other hand, Granick(9), in a recent review of iron metabolism, has suggested that hypoxia may influence iron absorption by an indirect effect on a humoral mechanism influencing the intestinal mucosa. Undoubtedly, hypoxia is capable of influencing iron metabolism very rapidly. In this respect, the studies of Huff and co-workers(10) are of interest. They found that plasma iron turnover rates and red cell iron renewal rates were increased to approximately twice their normal values after less than 12 hours at high altitude.

Another explanation of these findings is that the hypoxic stimulation of erythropoiesis sets off a humoral mechanism which is capable of causing increased gastro-intestinal iron absorption.

Summary. 1. Experiments with Fe^{59} were carried out to test the hypothesis that hypoxia

increases iron absorption. 2. Hypoxia was found to increase iron absorption by approximately one-third.

1. Stewart, W. B., Vassar, P. S., and Stone, R. S., *J. Clin. Invest.*, 1953, v32, 1225.

2. Copp, D. H., and Greenberg, D. M., *J. Biol. Chem.*, 1946, v164, 377.

3. Hahn, P. F., Bale, W. F., Ross, J. F., Balfour, W. M., and Whipple, G. H., *J. Exp. Med.*, 1943, v78, 169.

4. Dubach, R., Callender, S. T. E., and Moore, C. V., *Blood*, 1948, v3, 526.

5. Granick, S., *Bull. N. Y. Acad. Med.*, 1949, v25, 403.

6. Keller, J. G., *Am. J. Physiol.*, 1955, v183, 633.

7. Criscuolo, D., Clark, R. T., and Mefford, R. B., *ibid.*, 1955, v180, 215.

8. Vannotti, A., *Schweiz. med. Wschr.*, 1946, v76, 899.

9. Granick, S., *Bull. N. Y. Acad. Med.*, 1954, v30, 81.

10. Huff, R. L., Lawrence, J. H., Siri, W. E., Wasserman, L. R., and Hennessy, T. G., *Medicine*, 1951, v30, 197.

Received September 13, 1956. P.S.E.B.M., 1956, v93.

A Simple Method of Cutting Split Thickness Skin Grafts from Small Animals. (22799)

F. C. HANSEN AND C. A. HUBAY (Introduced by W. D. Holden)

Departments of Surgery, Western Reserve School of Medicine and University Hospitals, Cleveland, O.

The great increase in interest in homografting and especially in that of skin homografting prompts us to report what we have found to be a simple and reliable method of obtaining split thickness grafts from the rabbit and rat. Full thickness, pinch, and split thickness grafts all have their advocates and peculiar advantages. The split thickness graft, however, has been especially useful in our work for several reasons. 1) It has a high initial take (we have not yet had a failure). 2) Its hairlessness, softness and thinness make viability easy to evaluate, and also make it ideal for use in millipore chamber technics. 3) It requires no dressing of any kind.

Methods heretofore mentioned in the literature are either too cumbersome or require expensive equipment such as the Brown Electro dermatome. We have found the following method to be simple, rapid, and dependable. The equipment required is inexpensive and can be used by relatively inexperienced persons.

Method. In essence the skin to be used is stretched tightly over a flat block by means of a special clamp and a graft is cut freehanded.

In detail: 1) Donors are clipped closely and widely over the donor site and the remaining stubble is epilated with Barium Sulphide and corn starch paste. The donors are then kept for 1-3 days at which time a fine stubble of new hair growth will indicate the anlagen phase of the skin or hair growth cycle. 2) Under barbiturate anesthesia an incision is made at one end of the donor area and the skin and panniculus carnosa is elevated from the underlying fascia by blunt dissection. 3) The specially shaped block (Fig. 1) is introduced into the space thus formed and the clamp is applied so as to hold the skin stretched tightly over the block. 4) At this point it is advantageous to make any desired identifying marks by pricking the skin with a #24 needle dipped in India ink (Fig. 2). 5) The graft can now be cut easily with a straight razor held freehand. The clamp serves as a handle to steady the skin. Grafts of as much as 60 sq cm can be cut in a single sheet from a 300 g rat (Fig. 2). 6) The clamp is removed, the block withdrawn and the incision closed with Michel clips or may be sutured as desired. 7) The grafts may be applied to the recipient bed by any method. We have found Michel clips to be a very rapid and entirely satisfactory way of securing the graft (Fig. 3). 8) By placing the