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EFFECT OF DIETARY Fe-DEFICIENCY ON GROWTH ORGAN WEIGHT, HAEMOGLOBIN FORMATION AND Fe^{59}-RETENTION IN RATS

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Introduction

Fe-deficiency anaemia in growing children is well known. Rats show similar type of haematological picture as human[1]. This paper reports the findings of a study that shows the effect of ten-fold reduction of dietary Fe on Fe^{59} uptake, haemoglobin formation, PCV (Packed Cell Volume) per cent, growth rates and organ weights in rats in a short term (42 days) evaluation, under controlled laboratory conditions. Fe levels in the diets were so maintained, that the lower level would result in haemoglobin deficiency while the upper level would be sufficient to maintain normal haematological status. The findings had helped emergence of a picture that even in a hectic growing period, a deceptive normalcy look is presented, as far as growth and outward appearance are concerned, though there can be morbidity due to Fe-deficiency anaemia.

Materials and Methods

Eighteen albino rats (females) of Sprague Dawley strain, each weighing 43g. (43 ± 2g) was divided into two groups, each having nine rats. The rats were kept in specially devised perpex cages to avoid metal contamination. The corn meal diet[2] (basal diet) with all essential nutrients except Fe, was given *ad libitum* to the rats. De-ionized water was given to rats for drinking. The rats of group I were given Fe+++ at the level of 5 ppm only, whereas the rats of group II got it at 50 ppm level. The rats were weighed at the beginning of the experiments and then weekly and then prior to their sacrifice, on a top-pan Amoval balance which gives accuracy to the level of decigram. The rats were starved for 24 hours on the 34th day of the experiment and after starvation, they were given one μ curie of $^{59}FeSO_4$ in five g of diet. Since they were starved, each of them consumed all five g diet given to them. Initial Fe^{59} count was taken after 12 hours of Fe^{59} administration and the final count after sevan days, taking of course, Fe^{59} counts intermittently within these seven days, to see body stabilisations of Fe^{59} activity. Counts were taken by a whole body gamma counter (Packard-Multi Channel Analyser) by keeping each rat vertically

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in a specially devised perpex box. On 42nd day's morning, the rats were made unconscious by chloroform, blood from heart of each rat was taken by disposable heparinized syringe. After the withdrawal of blood, each rat was sacrificed by gilloutine. Some organs, such as liver, kidney, heart, spleen etc. were separated out, blotted dry and then weighed in a top pan mettler balance having digital read out. Haemoglobiu was estimated by Drabkin's method[3] and PCV per cent was determined by micro-capillary method[4], centrifuging the blood in micro capillaries in a haematocrit centrifuge (one end of each capillary was blocked by plasticin) and the miniscus of the fluid and of the packed cell were read by an international microcapillary reader (Int. Equip. Co., USA).

TABLE I Haemoglobin (g/100 ml) and Packed Cell Volume (PCV) Percentage of Rats (Average values ± S.D.)

Estimations	Group I Fe - 5 ppm	Group II Fe - 50 ppm	Remarks
Haemoglobin (Hb) g / 100 ml.	7.34 ± 0.23	13.88 ± 0.19	Statistically significant $P < 0.01$
Packed Cell Volume (PCV) Percentage (%)	27.88 + 0.76	43.82 + 0.63	Statistically significant $P < 0.01$

Results and Discussion

Table I shows haemoglobin (Hb) level and Packed Cell Volume (PCV per cent) in the rats. Group I rats were severely anaemic-their haemoglobin level was reduced by around 47 per cent as compared to that of group II rats which had normal haemoglobin level. PCV per cent showed similar trend as indicated by a reduction by 36.4 per cent in group I rats with respect to group II rats.

Figure 1 shows the growth curves of group I and group II rats. Since they were weaning rats, they had hectic growth, which is indicated from the very sharply rising growth curves. The body weights of the group II rats were higher by 8.85 per cent than that of group I rats on 42nd day of experiment. Sharply rising growth curves, with no remarkable difference in slopes of the curves, indicated very good rates of growth in rats of both the groups. Outwardly the rats of both the groups could not be distinguished from each other, neither in physical appearance nor in agility within the cages. The rats of both the groups had similar food or drink consumption. It therefore appeared that even highly anaemic rats (Fe-deficiency anaemia), having lower physical activity

would have good growth rates and normal outward appearance or in other words, a deceptive look of normalcy.

TABLE II **Organ Weight**
(Average values ± S. D.)

Organ	Average Organ Weight/rat	
	Group I (g) ± S. D	Group II (g) ± S. D.
Liver	5.58 ± 0 280	6.373 ± 0. 570
Kidney	0.531 ± 0.025	0.592 ± 0 0532
Spleen	0.575 ± 0.081	0.574 ± 0. 270
Heart	0.738 ± 0.050	0.716 ± 0. 090

Table II shows the average organ weights as well as average organ weights/g of body weight for both the groups. In terms of average organ weights, group II (Fe-sufficient) rats had higher liver (by 12.44 per cent) and kidney (by 10.3 per cent) weights, with respect to group I (Fe-deficient) rats. Higher heart weight (by three per cent) was observed in group I rats with respect to group II rats. No difference in spleen weight was indicated. The above higher weights of organs for group II rats were just

Average Organ Weight/of Body Weight of Rat

	Group I (mg) ± S. D.	Group II (mg) ± S. D.	Remarks
Liver	31.7 ± 0.81	32.5 ± 1.94	Diff. not Significant
Kidney	2.95 ± 0.09	2.97 ± 0.13	-do-
Spleen	3.27 ± 0.44	2.93 ± 0 20	Significant
Heart	4. 2 ± 0.27	3. 6 ± 0.23	Significant $P<01$

the consequence of this higher body weight. But in order to judge whether there has been any effect in organ weight due to iron-deficiency, organs weight per gram of body weight should be the correct index. In terms of organ weight/g of body weight, it was seen that there was no significant difference in liver and kidney weights between the rats of two groups, whereas heart of group I rats seems to be remarkably enlarged and the spleen is also enlarged to some extent. Therefore, so far as the functions of these vital organs are concerned, liver and kidney did not seem to be under strain, whereas the heart and to some extent the spleen of Fe-deficient rats had been under strain to cope with haemoglobin deficiency and related physiological difficulties. The results are in consonance with previous findings[5],[6] except for liver where[6] enlargement of liver was indicated in case of marked anaemia in infants. It is inferred that when diets are adequate in all respects except Fe, liver is not affected.

TABLE III Fe^{59} Retention of Rats in Liver, Whole Blood and Whole Body (Average Values ± S.D.)

Item	Group I (Fe-5 ppm)	Group II (Fe-50 ppm)	Remarks
Liver	26.06 ± 188	24.06 ± 1.86	Statistically significant $P<0.01$
Whole blood	78.70 ± 9.36	22.67 ± 2.93	Statistically significant $P<0.01$
Whole body Count per minute (CPM) per rat	83153 ± 580	44869 ± 490	Statistically significant $P<0.01$
CPM per gm of body of the rat	472.89 ± 37.78	235.29 ± 31.97	Statistically significant $P<0.01$
Percentage (%) retention	51.64	27.86	Statistically significant $P<0.01$

Keeping all other nutrients at adequate and balanced proportions, except Fe, the present findings showed that Fe-deficiency alone in growing rats had brought about drastic reduction in haemoglobin and PCV per cent that is, the rats had become severely anaemic. Fe^{59}-uptake supported this picture.

Table III shows the whole body counts, the liver counts and the whole blood counts of Fe^{59} activity in the two groups of rats. It is evident that the retentions of Fe^{59} in the whole body, liver and whole blood were much higher in the rats of group I (Fe-deficient) when compared with group II (Fe-sufficient), indicating much higher uptake or absorption in group I, which, in turn, indicated the Fe-storage in group I rats was considerably depleted due to lower intake of Fe with the diet for a prolonged period. This clearly points out that during the growth period, the stored Fe can be depleted if Fe- intake is insufficient, keeping all other nutrients at the adequate levels. Both the groups were given the same dose of Fe^{59} (0.1 μ curie of Fe^{59}/5g of the diet/rat), yet the Fe^{59} retention, hence absorption was considerably more in group I, which indicated that Fe-up take (absorption and then retention) was need based, but not dose or supply based.

As it is well known that Fe-deficiency anaemia is hypochromic; microcytic, reduction in PCV per cent may be taken as an

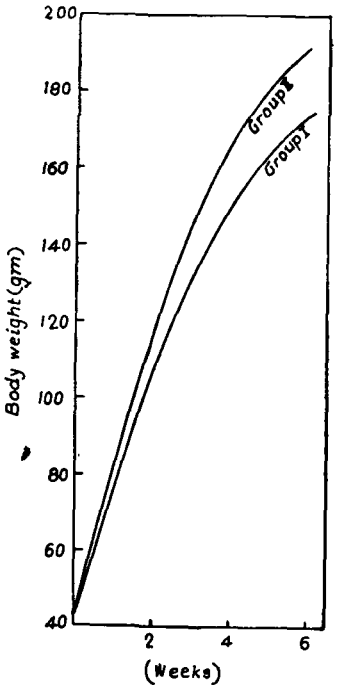
indication in the diminution of the RBC size, if RBC number is taken to be almost the same in both the groups (smaller RBC number could also result in Fe-deficiency anaemia if anaemia is moderate to severe[7]).

In the cage, the rats did not have the scope of performing much physical exercise, or in other words they were more or less in a sedentary state. Growth curves showed that under such conditions there was no striking difference in the actual growth. There was no difference in outward appearance too. That means that even for moderately anaemic subjects, in growing periods, the outward look as well as body weight would give deceptive normalcy, that is, would fall to indicate the actual morbidity, especially under conditions when they have lower level of physical activity.

It comes out that so far as Fe-deficiency anaemia is concerned, it is not the growth or outward look that can be used as an index of normalcy; the actual morbidity can be ascertained only from the blood picture.

Summary and Conclusions

Rats of the experimental group consuming diets adequate in all respects, but deficient in iron (5 ppm iron in contrast to 50 ppm iron for the control group) were found to suffer from moderate to severe anaemia (Hb 7. 39 ± 0. 2 g/100 ml, in contrast to 13.88 ± 0. 19 g/100 ml for the control group; PCV per cent 27.88± 0. 76 in contrast to 43.82 ± 0.65 for the control group) whereas they had satisfactory growth rates and normal outward appearance in all respects. However, their spleen and heart weight/g of body weight were also found to be significantly reduced, though liver and kidney weights remained normal Their Fe^{59} retention was considerably more than that of the control group indicating iron absorption and uptake were entirely dependent on iron-storage or haemoglobin status of the body. The results indicated that even for almost normal growth and a deceptive normal outward appearance a subject could be actually morbid because of Fe-deficiency anaemia under conditions of otherwise adequate diets and sedentary activity.



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