EFFECT OF VITAMIN B_{12} ON BLOOD AND TISSUE REGENERATION IN PHENYLHYDRAZINE INDUCED HAEMOLYTIC ANAEMIA

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Received on June 28, 1960

Abstract

Oral administration of Vitamin B_{12} is found to exhibit hemopoietic activity in hemolytic anaemia induced by Phenylhydrazine. The vitamin also counteracts the depressent effect of this chemical on leucocyte production.

Histological studies of liver and kidney in this hemolytic anaemia confirm the important role Vitamin B_{12} in tissue regeneration.

The significance of these findings lies in the possible clinical usefulness of Vitamin B_{12} in various types of hemolytic anaemias and toxic cellular necrosis.

Introduction

Vitamin B_{12} exhibits a large number of important and apparently diverse metabolic activities. It is essential for normal growth and nutrition. Cyanocobalamine is intimately related with the normal haemopoiesis by virtue of its role in nucleic acid and nucleoprotein synthesis (Robson and Keele, 1956), an impairment of which can cause macrocytic megaloblastic anaemia. The vitamin is not only essential for normal maturation of erythroblasts but also for the maturation of epithelial cells. A lipotropic effect (Gyorgy and Rose, 1950 and Drill and McCormic, 1946) is exhibited by this vitamin, which is known to prevent or correct the fatty infiltration of the liver caused by the deficient diets.

In previous studies (Chiplunkar *et. al.* 1957 and 1958) we have shown that acute haemolytic anaemia, induced by phenyl-hydrazine (12 mg/100 g) in rats, is of a macrocytic type in the earlier stages, and is associated with pathological lesions in liver, spleen and kidney. Since vitamin B_{12} facilitates normal blood and tissue regeneration, the influence of this vitamin on some of these pathological lesions in the acute haemolytic anaemia has been studied and reported in this communication.

Haematology

Experimental. The selection of animals and the procedure adopted to produce acute haemolytic anaemia, associated with liver and tissue injury, has been described earlier (Chiplunkar *et. al.* 1958). The anaemia was produced by

the intraperitoneal injection of phenylhydrazine, 12 mg./100 g. body weight. Vitamin B_{12} was administered orally, 5γ per animal, from the day of injection, in order to investigate its role both in preventing haemolysis of blood and in its haemopoietic activity during the development of anaemia. The control group did not receive any vitamin. The number of animals in each group were twelve. The animals were maintained on standard laboratory diet '*ad lib*'. The weights of the animals and the haematological changes were noted, at various periods, during the course of anaemia. The constituents determined were :—

(1) Erythrocytes, (2) Leukocytes, and (3) Haemoglobin.

Results. The overall changes in the body weights of the two groups are shown in Fig. I. The supplementation of vitamin B_{12} has lessened the initial

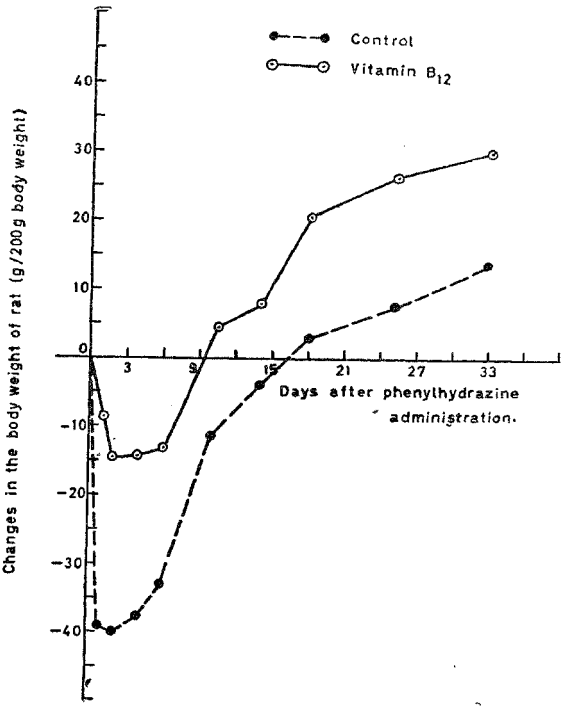

FIG. I

loss of weights and also has promoted the rate of growth as compared to the controls (Fig. I).

The haematological data are represented graphically in Fig. II. It is evident from this figure that vitamin B_{12} has not in any way lessened the initial haemolysis of blood by phenylhydrazine. But it has exerted a certain degree of haematopoietic response, as could be seen by the quicker regeneration and early attainment of normalcy of erythrocytic level in 33 days, as against the 42 days for the control group, to reach the same level.

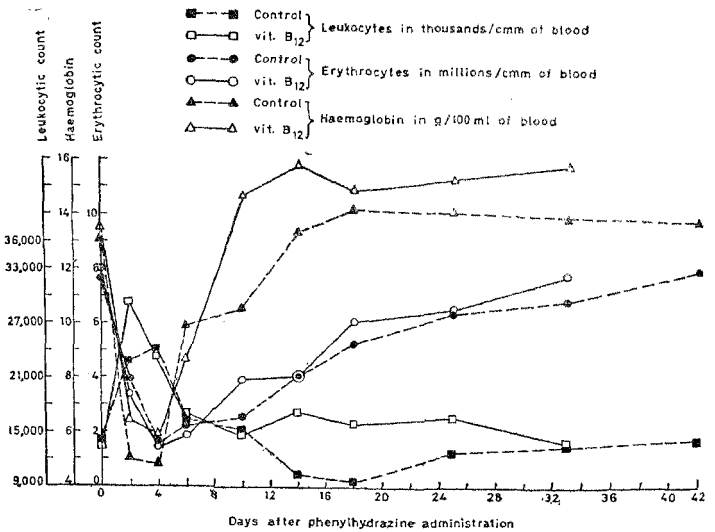

FIG. II

Vitamin supplementation has no effect on the initial fall in the haemoglobin concentration, as both the groups exhibit the same degree of decrease in haemoglobin level. However, B_{12} has influenced quicker synthesis of the haemoglobin. The normalcy in this group was attained by 9 days, in contrast to the 13 days required for the controls. Its influence is further marked by the higher level of haemoglobin maintained in B_{12} treated animals for quite an appreciable time.

The initial leukocytosis, after phenylhydrazine, is more pronounced in vitamin B_{12} rats and no depression of leukocytosis is observed at a later stage as seen in the control animals.

In general, it appears that the depressant effects of phenylhydrazine on the bone marrow functions has been considerably reduced by vitamin B_{12}.

Histopathology. The animals, three in number at each time, were killed by decapitation, at various intervals after phenylhydrazine administration and the tissues, *viz*., liver, spleen and kidney, were excised. For histopathological studies, tissue pieces were fixed in Bouines fluid, embedded in paraffin, sectioned and stained with haematoxylin and eosin.

Results. The photomicrographs of the representative types of lesions observed in the liver, spleen and kidney prior to and after administration of B_{12} are shown in plate I. The types of lesions observed in untreated animals have been described in detail earlier (Chiplunkar *et. al.* 1957). In contrast to these lesions, in the vitamin B_{12} treated group the pathology observed was much milder and regeneration rapid.

Liver. 3 days: In vitamin B_{12} treated rats, the fatty degeneration of parenchymal cells was minimal, the sinusoids were normal and diffused distribution of pigment was present. Few scattered cells of the parenchyma exhibited mitotic activity (Plate II). In the untreated animals heavy pigment deposit, dilatation of the sinusoids and definite fatty degeneration of cells were the lesions at this period (Plate I).

By 7th day in B_{12} rats the liver cells were practically normal in appearance. The pigment deposition was considerably lessened, and by 14th day no evidence of liver damage was seen. In the untreated animals, extensive fatty degeneration continued upto 14 days and the recovery was complete only after 20 days (Chiplunkar *et. al.* 1957).

Kidney. Vitaman B_{12} *supplemented group*, 3 days: There was slight tubular damage. Evidence of the deposition of pigment was not observed. Early signs of degeneration of the malpighian corpuscles were noticed by the presence of distended vascular tufts. After 7 days (Plate III) except for the occasional pigment deposition and the splitting up of the vascular tufts, a fairly normal pathology in the rest of the tissues was observed, in contrast to the control group wherein extensive deposition of pigment and early stage of glomerulonephritis were noticed (Chiplunkar *et. al.* 1957) (Plate IV). At the end of 21 days, the tissue was observed to be almost normal in the treated group, however, at this period, in control animal scattered deposition of pigment was noted.

Spleen. 3 days: B_{12} *treated animals*: Vascular congestion of the sinusoids was present, pigment deposition was seen, lymph corpuscles were showing evidence

of degeneration at some places. After 7 days the congestion was confined to the medulla and the cortex was free. The deposition of pigment had become extensive. After 14 days the congestion had almost disappeared and the pigment deposition was localised along the perimalphighean areas only (Plate 5). The splenic tissue returned to normalcy after 24 days. This type of damage to the splenic tissue was almost similar to the control group (Chiplunkar *et. al.* 1957).

DISCUSSION

The earlier regeneration of erythrocytes is definitely indicative of the stimulatory effect of the vitamin on erythropoiesis. Though it is generally accepted that B_{12} absorption is naturally poor by the gastro-intestinal tract, large doses are known to cause good haematological response. In the dose administered 5γ per animal equivalent to administering 1,000γ for an individual of 40 kg., the vitamin appears to have been absorbed and influenced blood regeneration.

Vit. B_{12} reduces the extent of damage to the liver tissue and also assists quicker regeneration. It is possible that B_{12} acts as a detoxifying agent to phenylhydrazine or its breakdown products, as do the cystine and methionine in chloroform poisoning (Himsworth, 1950) and thus prevent the extent of initial damage. By its effect on hemopoiesis, the period of hypoxaemic stage is lessened and the tissue recovery is hastened. Besides, our studies on the biochemical changes in phenyl-hydrazine hemolytic anaemia has revealed that in B_{12} treated rats depletion of glycogen in the liver is considerably less and the lipid content is not much enhanced as is the case with the control group.

Since the pathological lesions and the biochemical alterations in the liver of phenylhydrazine administered rats simulate those caused by many chemical poisons, synthetic and natural, it is possible that Vitamine B_{12} may also be found to be therapeutically useful in all these conditions of toxic necrosis of the liver.

SUMMARY

Vitamin B_{12} in larger doses exhibits hemopoietic activity when administered by the oral route in hemolytic anaemia induced by phenyl hydrazine. Vitamin B_{12} may, by quicker regeneration of the erythrocytes reduces the anoxaemic damage to the various organs and thus assist in recovering to the normal state in a shorter period. It might prove therapeutically useful in all hemolytic conditions whether of toxic, congenital or allergic origin. Its lipotropic effect is, in addition, a valuable adjunct in conditions of liver necrosis, particularly due to toxic agents. While facilitating erythropoiesis, the vitamin also seems to counteract the depressant effect of phenylhydrazine on leukocyte production. Whether this property could be utilized in conditions of granulocytopaenia of toxic origin in general, remains to be established.

Many aspects of the physiological actions of Vitamin B_{12} in the system are far from clear. But its role in the nucleic acid, protein, fat and carbohydrate

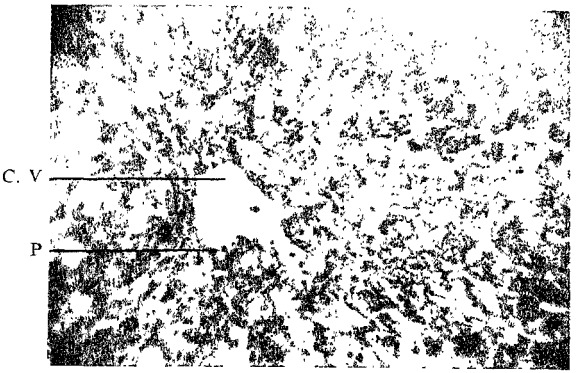

PLATE I

Rat - Liver - 7 days (control)

The fatty degeneration around the central vein and the fine granular nature of the lipoid material may be noted. Deposition of pigment is also marked.

H & E × 250; C.V. — Central vein; P — Pigment

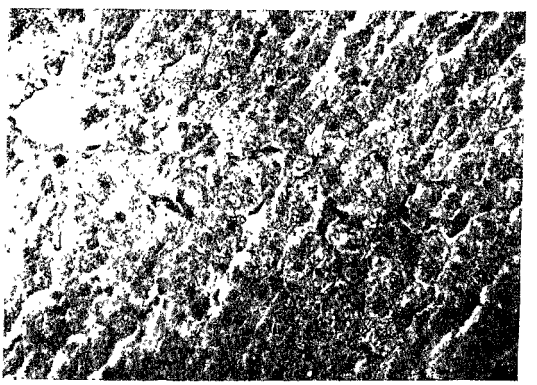

PLATE II

Rat - Liver - 7 days : Vitamin B_{12}

Liver cells are normal except for slight fatty change and scanty deposition of pigment. Attempts at regeneration of liver cells are seen.

H & E × 340

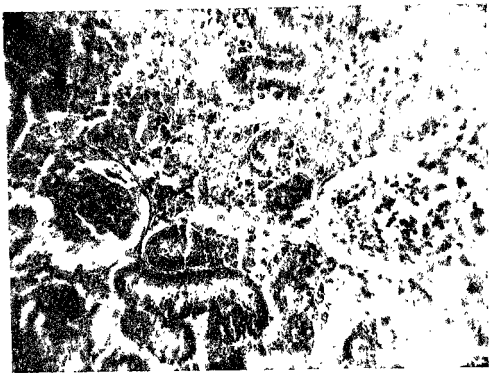

PLATE III

Rat - Kidney - 7 days (control)

Early signs of glomerulo-nephritis showing splitting of vascular tufts in reral tubes. The tubules are filled with hyaline material.

H & E. × 250

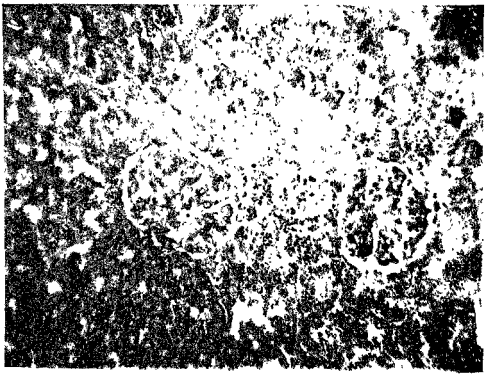

PLATE IV

Rat - Kidney - 7 days : Vitamin B_{12}

Kidney is normal except for the post-evidence of tubular degeneration. Glomeruli are becoming normal. Pigment is scanty.

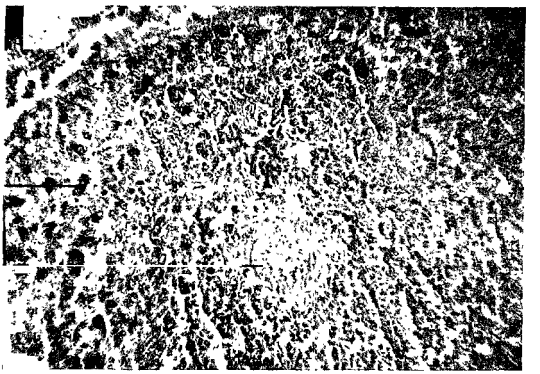

PLATE V

Rat - Spleen - Vitamin B_{12}

The perimalpighian distribution of pigment may be noted Splenic corpuscles are atrophied in some places. Follicular artery is slightly thickened.
H & E × 250; P — Pigment; F — Follicular artery

metabolism has established its importance in the fundamental cellular processes responsible for growth and maintainence of general health. Our studies on the liver and kidney in the pnenyl-hydrazine anaemia confirm the important role of Vitamin B_{12} in tissue regeneration. The significance of these findings lies in the possible clinical usefulness of Vitamin B_{12} in the various types of haemolytic anaemias and in toxic cellular necrosis.

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