

Observations on Serum B₁₂ and Folate Activity in Normal and Leukemic Rats

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DURING STUDIES on the induction of myelogenous leukemia in the rat, serum B₁₂ and folate activities were investigated. In man it is well known that serum B₁₂ activity is greatly elevated in chronic myelogenous leukemia (CML).¹ Less is known concerning serum folate activity but from initial reports and in our own experience, serum folate levels are usually normal or reduced in patients with leukemia.² The purpose of this study was to determine serum B₁₂ and folate activity in normal rats and rats subjected to total body radiation, oral administration of 3-methylcholanthrene (3MCA) and a combination of these agents. Of special interest were studies of serum B₁₂ and folate activity in rats with induced myelogenous and lymphatic leukemia as well as in rats with transplanted chloroleukemia (Shay).³

METHODS AND MATERIALS

Four ml. of whole blood were drawn by cardiac puncture while the animal was anesthetized, the blood was centrifuged and the serum removed and kept frozen until assay. Serum B₁₂ activity was measured by a Euglena assay method⁴ with various slight modifications introduced at the Thorndike Memorial Laboratory in the past 3 years and using an incubation time of 6 to 7 days. Results were expressed as $\mu\mu\text{g./ml.}$ of serum. Serum folate activity was determined by the use of *Lactobacillus casei* employing a technic somewhat modified by Herbert.⁵ In this study the incubation time was increased from 16–18 hours to 20–22 hours. Results were expressed in $\text{m}\mu\text{g./ml.}$ of serum.

Animals studied were of three breeds, standard non-inbred Wistar, inbred Furth-Wistar⁶ and an inbred Osborne-Mendel (Yale-Columbia) strain.⁷ Except for the Osborne-Mendel colony, all rats were maintained on normal diets throughout these studies. Rats of varying age groups and sex were included and observations were carried out on animals exposed to 450 r total body x-ray, on those given 180 mg. 3 methylcholanthrene (3MCA) and a combination of these agents. Only one group of rats was maintained on a special diet; the Osborne-Mendel strain were fed a vitamin A deficient diet consisting of whole wheat and dried milk powder. The findings in this group of animals were especially noteworthy.

RESULTS

Serum B₁₂

A series of 43 normal rats were found to have serum B₁₂ levels similar to humans; the mean value was $364 \pm 141 \mu\mu\text{g./ml.}$ with a range of 154 to 840 $\mu\mu\text{g./ml.}$ (table 1). The great majority had levels between 150 and 500 $\mu\mu\text{g./ml.}$; only three rats had values of 500 to 840 $\mu\mu\text{g./ml.}$ In this series the

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Table 1.—Serum B₁₂ Activity in Normal and Leukemic Rats ($\mu\mu\text{g./ml.}$)

Group Studied	No. Rats	Mean	Standard Deviation
Normal	43	364	± 141
Osborne-Mendel*	13	82	± 41
450 r x-ray	31	316	± 162
3MCA	12	542	± 330
Chloroma (Shay)	19	362	± 166
Pulmonary infection	4	171	± 7
Acute lymphatic leukemia	2	1875	± 672
Myelogenous leukemia	8	329	± 194

*Maintained on vitamin A-deficient diet.

mean values were not influenced by either age or sex. The only significant variation from normal values was found in the Osborne-Mendel rats. These animals were found to have low serum B₁₂ levels with a mean of $82 \pm 41 \mu\mu\text{g./ml.}$ and a range of 20 to 150 $\mu\mu\text{g./ml.}$ for the 13 rats studied. None of these rats displayed hematologic evidence of B₁₂ deficiency. Following total body radiation (450 r), serum B₁₂ determinations on 31 rats showed a mean of $316 \pm 162 \mu\mu\text{g./ml.}$ with a range of 148 to 1162 $\mu\mu\text{g./ml.}$ There was no demonstrable effect of time of radiation on B₁₂ level, i.e. determinations on serum drawn 1 to 2 days following radiation showed no significant differences from those drawn 3 to 4 months later. In rats given a total dose of 180 mg. of 3MCA by stomach tube over a period of 18 days, the serum B₁₂ values were $542 \mu\mu\text{g./ml.} \pm 330$ with a range of 212 to 1320 $\mu\mu\text{g./ml.}$ This group of animals showed somewhat elevated serum B₁₂ values but the number of animals was small and the significance of these findings is questionable. During this investigation four rats developed a leukocytosis secondary to a severe pulmonary infection. These rats had low normal serum B₁₂ levels, mean 170 $\mu\mu\text{g./ml.}$ with a range of 164 to 188 $\mu\mu\text{g./ml.}$

In addition to the above studies, observations were carried out on 19 adult non-inbred Wistar rats bearing large transplanted chloromas (Shay). These animals had a mean of 362 ± 166 with a range of 190 to 822 $\mu\mu\text{g./ml.}$ Of particular interest were the investigations on rats with induced leukemia. Three animals developed acute lymphatic leukemia following 450 r total body radiation and in two, serum B₁₂ and folate levels were obtained. After discovery of the leukemic blood picture, the rats became ill very rapidly and were sacrificed. Serum B₁₂ levels were 2160 $\mu\mu\text{g./ml.}$ and 1440 $\mu\mu\text{g./ml.}$, and the determinations were checked by two separate assays. Thus far in these experiments, nine rats have developed myelogenous leukemia; six following total body x-ray, two after 3MCA and one following x-ray and 3MCA. Details of the cytologic, histologic and other findings on these rats will be reported. Of nine animals with myelogenous leukemia, B₁₂ and folate levels were obtained on eight at the time of sacrifice (see table 3). Cytologic and histochemical studies established the myeloid character of the leukemia, but strict classification into acute or subacute forms was not possible.³ Typical green chloromatous tissue was found at post-mortem examination in five of the nine cases. One rat (#351) presented the peripheral blood picture of

Table 2.—Serum Folate Activity in Normal and Leukemic Rats ($m\mu\text{g./ml.}$)

Group Studied	No. Rats	Mean	Standard Deviation
Normal	100	129	± 27
Osborne-Mendel*	10	78	± 20
450 r x-ray	11	144	± 35
3MCA	8	132	± 20
Pulmonary infection	5	148	± 40
Chloroma (Shay)	16	168	± 33
Acute lymphatic leukemia	2	62	± 3
Myelogenous leukemia	8	86	± 35

*Maintained on vitamin A-deficient diet.

chronic myelogenous leukemia but terminally the disease resembled a more subacute form.

Serum Folate

At the time of these studies values for serum folate activity in the rat (using the *L. casei* technic) had not appeared in the literature. Therefore, to establish normal values, determinations were carried out on 100 inbred Furth/Wistar rats. The mean serum folate activity of this group was $129 \pm 27 m\mu\text{g./ml.}$ with a range of 55 to $190 m\mu\text{g./ml.}$ These values are in marked contrast to human serum folate levels of 7 to $25 m\mu\text{g./ml.}$ Studies of serum folate activity were carried out on groups of rats under a variety of conditions, but, as shown in table 2, the slight differences noted were of questionable significance.

DISCUSSION

While relatively few animals were studied, there was no apparent influence on serum B_{12} by x-ray or 3MCA. The low serum B_{12} activity found in the Osborne-Mendel rats was of considerable interest. These rats were descendants of the colony described in 1936 by Wilens and Sproul⁷ who were the first to describe the occurrence of leukemia de novo in the rat. These authors found at autopsy among 361 old Osborne-Mendel rats maintained on several special diets, 11 unequivocal cases of myelogenous leukemia. Whether the high incidence of myeloid leukemia in these animals was related to dietary deficiency or to genetic factors or both, remains to be determined.

In humans, serum B_{12} activity is greatly elevated in chronic but not in acute myelogenous leukemia. Investigation of the B_{12} levels was carried out on rats with leukemia induced by x-ray and 3MCA in this study. However, since all but one of the myelogenous leukemias were acute or subacute, it is not surprising that elevated serum B_{12} levels were not encountered. Rat 351 presented a blood picture of CML, but as the disease progressed the disease became more subacute in character. The serum B_{12} activity in this rat at the time of sacrifice was $740 \mu\text{g./ml.}$, a high normal value (see table 3).

Three rats in this series developed acute lymphatic leukemia following 450 r total body x-ray. Serum B_{12} activity was determined in two of these

Table 3.—Serum B₁₂ and Folate Activity in Leukemic Rats

Rat No.	Sex	Agent	Diagnosis	WBC	B ₁₂	F.A.
351	M	3MCA	CML	665,000	740	122
562	M	450 r	acute ML	31,000	426	65
224	F	3MCA	subacute ML	139,400	372	140
451	F	450 r	subacute ML	56,000	325	94
466	F	450 r	subacute ML	44,000	203	95
385	F	450 r	subacute ML	174,000	115	70
		3MCA				
439	M	450 r	acute ML	50,300	205	30
470	F	450 r	subacute ML (basophilic)	82,000	249	75
484	M	450 r	acute LL	69,800	1400	59
566°	M	450 r	acute LL	338,000	2349	63

*Furth-Wistar rat; all others non-inbred Wistar rats.

animals and revealed elevated levels (table 1). However, at autopsy, severe necrosis of liver cells was found in these animals and no doubt accounted for the high serum B₁₂ activity. Liver damage of this sort was not present in the rats with myelogenous leukemia.

The serum folate activity, by the *L. casei* method used in this study, was found to be much higher in the rat than in man. However, the folate levels were not significantly altered in various groups of rats studied. The moderate reduction of serum folate activity noted in leukemic rats and Osborne-Mendel rats on vitamin A-deficient diet are of questionable significance since only small numbers of animals were investigated.

SUMMARY AND CONCLUSIONS

In normal rats serum B₁₂ activity was found to be similar to man but serum folate activity was a good deal higher, in the range of 129 mμg./ml. Total body radiation and administration of 3MCA did not significantly alter B₁₂ or folate activity. Osborne-Mendel rats maintained on a vitamin A-deficient diet were found to have markedly lowered serum B₁₂ activity; the folate levels were only slightly decreased.

Studies on leukemic rats showed that eight animals with myelogenous leukemia had no elevation of the serum B₁₂ activity, and in these animals folate levels were slightly reduced. In two rats with radiation-induced acute lymphatic leukemia, serum B₁₂ activity was significantly elevated due to hepatic necrosis, and folate values were somewhat below normal levels.

SUMMARIO IN INTERLINGUA

In rattos normal, il esseva trovate que le activitate seral de vitamina B₁₂ esseva simile a illo in le homine, sed le activitate seral de folato esseva marcatamente plus alte, in le cordine de 129 mμg per ml. Irradiation del corpore total e administration de 3-methylcholanthreno non alterava significativemente le activitate de vitamina B₁₂ o de folato. Esseva constatate que rattos Osborne-Mendel, mantenite con un dieta a carentia de vitamina A habeva un marcate-

mente activitate seral de vitamina B₁₂. Le nivellos de folato esseva reducite solo levemente.

Studios in leucemic rattos monstrava que octo animales con leucemia myelogene habeva nulle augmento del activitate seral de vitamina B₁₂, e in iste animales le nivellos de folato esseva levemente reducite. In duo rattos con acute leucemia lymphatic inducite per irradiation, le activitate seral de vitamina B₁₂ esseva significativamente elevate in consequentia de necrosis hepatic, e le valores pro folato esseva levemente infra le nivellos normal.

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