Further Observations on Polycythemia in Hepatocellular Carcinoma

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AN ASSOCIATION between hepatocellular carcinoma and polycythemia was reported in 1958 by McFadzean, Todd and Tsang[1] who found the red cell mass to be increased in 17 of 28 patients investigated. The red cell masses were calculated from the plasma volumes determined by the T-1824 dye method. When radioactive isotopes became available it was decided further to investigate the red cell mass in hepatocarcinoma by direct measurement with Cr^{51} tagged red cells and to determine whether an erythropoietic stimulating factor was demonstrable in the plasma of the patients with polycythemia.

MATERIAL AND METHODS

In 1960, 51 patients suffering from hepatocellular carcinoma were admitted to the wards of the University Department of Medicine. An unrelated investigation rendered 12 of these patients unavailable for this study. Of the remaining 39, 19 at the time of their admission were in the terminal phase of their illness, were pyrexial, had anasarca or had suffered recent gastrointestinal hemorrhage, thereby rendering them unsuitable for this investigation. Observations were therefore made on the 20 patients remaining. There were 17 males and 3 females with a mean age of 45.7 $\pm$ 8.2 years. The duration of symptoms prior to admission ranged from 1 to 7 (mean = 2.9 $\pm$ 1.7) months, and death occurred from 2 weeks to 6 months (mean = 1.7 $\pm$ 1.3 months) after admission. All had massive, palpable tumors of the liver. The diagnosis of hepatocellular carcinoma was confirmed at necropsy or by aspiration needle biopsy. Clinically it was held in all cases that the tumor had arisen in a cirrhotic liver and this was confirmed at necropsy in 18 instances, the cirrhosis being classified as post-necrotic. None of the patients had clinically detectable anasarca at the time the studies were made.

Twelve male and 3 female patients without hematologic abnormality and disturbance of water and electrolyte balance served as controls for the blood volumes. Forty male and 20 female medical students were investigated to determine the hemoglobin, venous hematocrit, reticulocyte, total leukocyte and platelet values in the peripheral blood of healthy Chinese.

Hemoglobin was determined by the method of Sanford, Sheard and Osterberg[2] and the venous hematocrit as described by Wintrobe.[3] Reticulocytes were counted after examining at least 1000 red cells in films vitally stained with brilliant cresyl blue and platelets were enumerated by the formol-citrate diluent method described by Dacie.[4] Sternal marrow smears were prepared according to Davidson.[5] The red cell mass was determined with Cr^{51} tagged red cells employing essentially the procdure described by Veall and Vetter[6] save that approximately 80 μc of Cr^{51} were added to 15 to 20 ml. of the patients' blood mixed with 5 ml. of acid-citrate-dextrose solution. The volume of blood employed was inversely proportional to the hematocrit. In each patient the plasma volume simultaneously was determined using T-1824. After injection of the labeled red cells and the dye, blood samples were taken at 10, 20, 30 and 40 minutes. The mean radioactivity of the 30 and 40 minute samples, read in a well-type scintillation counter, was employed in the calculation of the red cell mass. The T-1824 concentration in the plasma was determined in a spectrophoto-

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meter and the plasma volume calculated after extrapolation to zero time. The patients were recumbent and received fat-free breakfasts and no lunch before the test.

The assay of erythropoietic stimulating factor (ESF) in plasma by Fe^{59} incorporation into rat red cells is generally accepted to yield consistent results.[7] In the present investigation the method of Gurney and Pan[8] was employed, modified only in that the tracer dose of Fe^{59} was administered intraperitoneally as described by Naets.[9] Starved Wistar rats of about 150 Gm. in weight were used and each assay group consisted of 5 or more animals. Four patients with polycythemia associated with hepatocellular carcinoma were investigated. Four patients with aplastic anemia and 6 healthy subjects served as controls.

Van Slyke's apparatus was employed to determine arterial oxygen saturation in patients no. 13 to 17 (table 1), all of whom had polycythemia. These findings were compared with those in 5 patients with hepatocellular carcinoma with normal red cell masses.

The hemolytic index was determined as outlined by McFadzean, Todd and Tsang.[1] The mean of 25 healthy controls was 18 ± 1.4.

Standard statistical methods were employed throughout and the standard deviation is shown after each mean value.

Results

The red cell mass, plasma volume and other relevant data in each of the 20 patients investigated are set out in table 1. It will be seen that the red cell mass was increased in 11, normal in 8 and reduced in one. The plasma volume was increased in 15 and this expansion was independent of changes in the red cell mass. When compared with the controls, the male patients had a significantly increased mean red cell mass ($P < 0.001$) and mean plasma volume ($P < 0.001$). In the 3 female patients, the red cell mass was above normal limits in 2 and the plasma volume in all 3 was increased. Neither the hemoglobin nor the venous hematocrit levels afforded an accurate index of the red cell mass, largely because of the presence of expansion in plasma volume. Nevertheless, in general, the venous hematocrit rose with increase in the red cell mass and a venous hematocrit of over 48 per cent was invariably associated with an increase in that mass.

Normoblastic hyperplasia was present in the marrow of all the patients and tended to be more pronounced in those patients with polycythemia. There was no hyperplasia of the myeloid series nor of megakaryocytes. The mean reticulocyte count was 0.9 ± 0.8 per cent and although this was not a marked elevation, it was significantly higher than that of the controls, 0.2 ± 0.2 per cent ($P < 0.001$). The mean total leukocyte and platelet counts were 7090 ± 1654 and 189,050 ± 70,900 per cu.mm. respectively and were not significantly different from those of the healthy controls.

The results of the assay of ESF in plasma are set out in table 2. In the 4 patients with marked polycythemia associated with hepatocellular carcinoma, ESF was not increased. This was in contrast to the findings in 4 patients with aplastic anemia.

In 5 patients with polycythemia the mean arterial oxygen saturation was 93.2 ± 2.1 per cent. This was not significantly different from the findings in 5 patients (94.2 ± 2.0 per cent) in whom polycythemia was absent.

A mean hemolytic index of 24.0 ± 9.2 was obtained in 4 patients with polycythemia. This was not significantly different from that in 4 patients with

Table 1.—*Certain Hematologic Data in Patients with Hepatocellular Carcinoma*

	Hemoglobin (Gm. per 100 ml.)	Venous h'crit (per cent)	Red cell mass (ml. per Kg.)	Plasma volume (ml. per Kg.)	Total blood volume (ml. per Kg.)
Male patients					
1	9.55	28	17.8	53.7	71.5
2	11.40	36	28.6	60.0	88.6
3	12.45	38	32.8	57.7	90.5
4	13.35	39	26.6	60.5	87.1
5	12.85	40	33.2	61.8	95.0
6	13.55	43	31.6	51.0	82.6
7	13.00	44	30.0	52.2	82.2
8	12.00	44	36.6	60.2	96.8
9	13.55	44	40.7	61.1	101.8
10	16.20	45	33.5	48.4	81.9
11	14.75	48	43.1	55.8	98.7
12	14.35	50	46.1	56.6	102.7
13	16.20	56	46.5	44.0	90.5
14	14.80	56	52.0	58.0	110.0
15	17.20	60	63.0	58.7	121.7
16	20.65	69	75.0	45.1	120.3
17	21.30	70	70.5	42.2	112.7
Mean	14.53	47.6	41.6	54.5	96.1
S.D.	± 3.00	± 11.2	± 15.3	± 6.2	± 13.6
Male controls					
Mean	15.04	45.8	29.1	42.7	71.8
S.D.	± 0.87	± 3.1	± 3.4	± 5.9	± 8.2
Range	13.00–17.40	40–54	22.4–35.5	32.4–50.8	57.8–81.7
Female patients					
18	10.65	31	23.0	66.2	89.2
19	11.20	38	32.0	69.8	101.8
20	11.80	42	31.4	55.5	86.9
Mean	11.20	37.0	28.8	63.8	92.6
S.D.	± 0.51	± 4.5	± 4.1	± 6.1	± 7.0
Female controls					
Mean	13.21	41.7	25.1	41.5	66.7
S.D.	± 0.56	± 2.1	± 1.5	± 3.4	± 4.3
Range	12.05–14.00	36.5–46	23.4–27.0	37.3–45.6	62.3–72.6

normal red cell masses, 29.9 ± 17.4. However, the mean hemolytic index of these 8 patients was significantly higher than that in healthy controls, 18 ± 1.4 ($P < 0.001$).

In order to determine yet again the incidence of polycythemia in patients with hepatocellular carcinoma, the findings in all patients admitted with this disease from 1957 to 1960, subsequent to those referred to in the previous report,[1] were reviewed. It has been shown that patients with a venous hematocrit in excess of 48 per cent invariably have an increase in the red cell mass. Employing this criterion it was found that 17 of 145 patients were polycythemic, an incidence of 11.7 per cent which is not markedly different from that previously reported.[1]

Table 2.—*Plasma Erythropoietic Stimulating Factor in Polycythemia Associated with Hepatocellular Carcinoma and in Controls*

Subjects	Fe^{59} red cell incorporation (per cent of injected dose)
1. Controls	2.2 ± 0.5
2. Hepatocellular carcinoma with polycythemia:	
patient 12	2.6 ± 0.4
patient 13	2.9 ± 0.4
patient 16	2.5 ± 0.8
patient 17	1.7 ± 0.2
3. Aplastic anemia:	
patient A	45.5 ± 7.3
patient B	46.1 ± 9.7
patient C	45.7 ± 3.2
patient D	26.4 ± 5.6

Discussion

Direct measurement of the red cell mass confirms the findings of McFadzean et al.[1] of the occurrence of polycythemia in patients with hepatocellular carcinoma. In the 20 patients investigated, venous hematocrit levels of over 48 per cent were invariably associated with polycythemia. Employing this criterion, the over-all incidence of polycythemia in a second series of 145 patients with hepatocellular carcinoma was found to be 11.7 per cent which does not differ significantly from that in the series previously reported.[1]

Expansion of the plasma volume was present in 15 of the 20 patients and this is attributable to the associated cirrhosis of the liver.[1] It is of interest to note that the plasma volumes were within normal limits in the two most severely polycythemic patients. This might well be the result of an encroachment upon the plasma volume by the grossly increased red cell mass under circumstances in which no further expansion of the total vascular compartment occurs. The expansion of the plasma volume is responsible for the finding in certain patients of a low venous hematocrit in the presence of a normal red cell mass and of a normal hematocrit in the presence of an increased red cell mass.

If it is assumed that the onset of symptoms marks the initial appearance of the tumor, then the development of the polycythemia is remarkably rapid, for the duration of symptoms prior to admission to hospital ranged from 1 to 7 (mean = 2.9 ± 1.7) months. Further, the venous hematocrits reported persisted without significant change until death or until the hemorrhage, gastrointestinal or intraperitoneal, which heralded death.

Although the precise incidence of polycythemia vera in Chinese in Hong Kong is not known it would appear to be low. In the 4 years from 1957 to 1960, 4 such cases were seen in this Department in contrast to 17 patients with polycythemia associated with hepatocellular carcinoma. Polycythemia in hepatocellular carcinoma differs from polycythemia vera in that elevation of peripheral leukocytes and platelets in association with myeloid and megakaryocytic hyperplasia in the bone marrow were not encountered. Further, the sequence of events is known, for in 3 patients with cirrhosis of the liver ob-

served over a period of some years not included in this series, polycythemia appeared following the development of hepatocellular carcinoma. In view of the absence of thrombocytosis and leukocytosis, a term more appropriate than "polycythemia" would be "erythrocythemia" or "erythrocytosis".

In none of the patients was there any clinical evidence of the types of cardiac and pulmonary abnormalities associated with secondary polycythemia. It has been shown that the arterial oxygen saturation in the patients with polycythemia did not differ from that in the patients with normal red cell masses.

The polycythemia cannot be the result of a prolonged red cell life-span. In patients previously reported,[1] the mean hemolytic index was significantly elevated. In the 8 patients investigated in the present series it was also greater than normal and there was no difference btween those with polycythemia and those without.

It has been suggested that an etiologic factor in polycythemia vera may be an increased production or a decreased rate of destruction of ESF. This suggestion was based on the findings by certain workers of an increase of ESF in the plasma of patients with this disease. For example, ESF, as assayed by the Fe^{59} incorporation into rat red cells, was increased in many of the patients investigated by Contopoulos, McCombs, Lawrence and Simpson[10] and by Korst, Whalley and Bethell.[11] However, as has been emphasized by Gordon,[12] the attempts by other workers including himself to demonstrate such an increase have been uniformly unsuccessful. The possibility of an increase in ESF in the plasma having etiologic significance in the polycythemia of hepatocellular carcinoma required investigation. In the 4 patients investigated, 2 of whom had markedly increased red cell masses, no increase in ESF was found.

The development of polycythemia secondary to renal carcinoma and to uterine fibroids[13-18] is well established. The occurrence in the former condition has recently been reviewed by Damon, Holub, Melicow and Uson[19] and by Forssell.[20] The polycythemia encountered in patients with these tumors, like that with hepatocellular carcinoma, is not associated with leukocytosis and thrombocytosis. Removal of the tumor in many of the patients with renal carcinoma and in those with uterine fibroids was followed by disappearance of the polycythemia. As far as we are aware, plasma ESF has been determined in only one patient with renal carcinoma and polycythemia.[11] In this patient it was increased and the plasma ESF decreased when the polycythemia disappeared after removal of the tumor. Hewlett, Hoffman, Senhauser and Battle[21] found that extracts prepared from a renal carcinoma associated with polycythemia contained more ESF than normal renal tissue or a renal carcinoma not associated with polycythemia. On the other hand there is a report of one patient with bilateral ovarian carcinoma associated with polycythemia and in this patient filtrates of both the plasma and tumor failed to affect erythropoiesis in the rat.[12] Manifestly more patients with this form of "secondary" polycythemia must be investigated before conclusions may be drawn as to the relationships each with the other.

Summary

Direct measurement of the red cell mass has confirmed a previous report of the occurrence of polycythemia in patients with hepatocellular carcinoma. Twenty patients (17 males and 3 females) have been investigated. The red cell mass was increased in 11, normal in 8 and reduced in one. Because of hypervolemia, present in 15 of the 20 patients investigated and attributable to the associated cirrhosis of the liver, the hematocrit might be normal in the presence of an increased red cell mass. A venous hematocrit of 48 per cent and above was found invariably to be associated with an increase in the red cell mass. Using this criterion, 17 of 145 patients with hepatocellular carcinoma were found to be polycythemic, an incidence of 11.7 per cent.

Plasma erythropoietic stimulating factor determined by Fe^{59} incorporation into red cells of fasted rats was not increased in 4 patients with hepatocarcinoma and polycythemia.

These findings are briefly discussed.

Summario in Interlingua

Le mesuration directe del massa de erythrocytos ha confirmate un previe reporto del occurrentia de polycythemia in patientes con carcinoma hepatocellular. Vinti patientes—dece-septe masculos e tres femininas—esseva investigate. Le massa del erythrocytos esseva augmentate in dece-un, normal in octo, e reducite in un. A causa del hypervolemia que esseva presente in dece-cinque del vinti patientes investigate (e que esseva attribuibile al presentia associate de cirrhosis del hepate), le hematocrit pote ben esser normal in tal caso in despecto del augmentate massa de erythrocytos. Un hematocrite venose de 48 pro cento e plus se trovava invariabilemente in association con un augmento del massa de erythrocytos. A base de iste criterio il esseva trovate que dece-septe inter 145 patientes con carcinoma hepatocellular esseva polycythemic. Isto es un incidentia de 11,7 pro cento.

Le nivello de factor de stimulation erythropoietic, determinate per le incorporation de Fe^{59} in le erythrocytos de rattos jejun, non esseva elevate in quatro patientes con hepatocarcinoma e polycythemia.

Le signification de iste constatationes es discutite brevemente.

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