Increased Peripheral Oxygen Delivery in Thyrotoxicosis:

Role of Red Cell 2, 3-Diphosphoglycerate

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IT HAS become increasingly apparent that the amount of organic phosphates within the red cell is a primary influence on the position of the oxyhemoglobin dissociation or equilibrium curve and, therefore, the relative affinity of hemoglobin for oxygen. Of the organic phosphates within the red cell, 2, 3-diphosphoglycerate (2, 3-DPG) is quantitatively, most important, since it comprises 60 to 70 per cent of the total, with adenosine triphosphate making up most of the remainder. In 1967, Benesch and Benesch [2, 3] and Chanutin and Curnish [5] demonstrated, *in vitro*, that reversible binding of these substances to hemoglobin markedly influenced the equilibrium curve, and that the addition of 2, 3-DPG alone to hemoglobin solutions would move the curve "to the right" and thus facilitate the unloading of oxygen. These studies prompted our group, as well as others, to study the effect of several clinical states, characterized by an imbalance between oxygen supply and demand, on 2, 3-DPG levels and the resultant shifts in the oxyhemoglobin equilibrium curve. We now wish to report significant elevations of 2, 3-DPG levels in hyperthyroidism, resulting in increased oxygen release from red cells. We have further shown that the level of this substance is a sensitive indicator of the state of peripheral tissue metabolism in this disease and, moreover, that the circulating thyroid hormone, triiodothyronine (T_3), will directly induce increased production of 2, 3-DPG within the red cell.

Methods and Materials

Eight patients, who had entered the Hospital of the University of Pennsylvania for evaluation and treatment, were selected for the study. All had clinical signs and symptoms of thyrotoxicosis. All had laboratory studies confirming the diagnosis including a protein-bound iodine (PBI) determination or an ^{131}I uptake study, or both. Several had ^{131}PBI and T_3 determinations, as well.

In addition, one of the authors (W. W. M.) a healthy, 35-year-old man, administered triiodothyronine (Cytomel®) to himself, in doses of 200 μg./day for 2 weeks. Measure-

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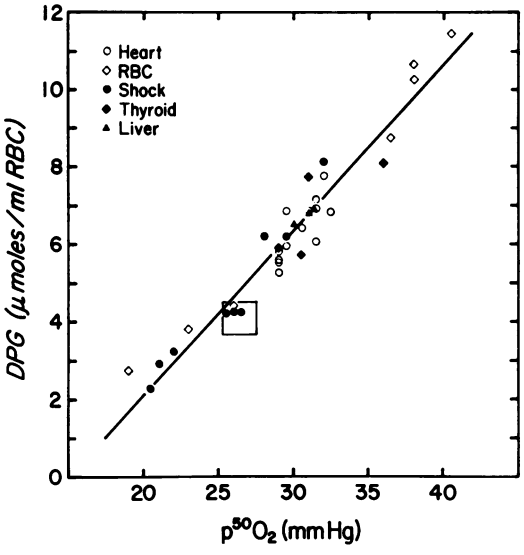

FIG. 1. Regression line of red cell 2, 3-DPG versus the P_{50} (position of the oxyhemoglobin dissociation curve). Normal values are within the central square. The correlation coefficient for this relationship was 0.97.

ments of thyroid function and oxygen consumption, by the closed technic, were performed before, during, and after the hyperthyroid state was induced. Typical symptoms began 4 days after beginning T_3 and disappeared 4 days after the drug was discontinued. As with the majority of the patients with spontaneous onset of the disease, these included heat intolerance, nervousness, hyperreflexia, tachycardia at rest and with exertion, and unusual loss of weight.

Determinations of 2, 3-DPG were performed by the Schroter[19] modification of the method of Krimsky.[16] The determinations were done on all patients before the beginning of treatment. Three patients were studied by serial determinations.

Oxygen-hemoglobin equilibrium was measured, using 20 ml. of heparinized venous blood in a variety of clinical conditions (Fig. 1). In each analysis, equal amounts of whole blood were equilibrated in two tonometers with 100% nitrogen and 100% oxygen, respectively. A constant volume of carbon dioxide was used to maintain pH at 7.345. After 20 minutes of equilibration, varying amounts of blood were withdrawn anaerobically from each tonometer and were mixed thoroughly with mercury, in oiled, 3 ml. glass syringes. For each analysis 3 to 6 blood mixtures were used to obtain oxygen saturation values ranging from 20 to 70%. Oxygen saturation was measured by direct light spectrophotometry. Oxygen tension was measured with the appropriate electrode at 37° C. using an Instrumentation Laboratory pH/gas Analyser, Model 113. Duplicate determinations were made for each point on the curves. The oxygen tension of whole blood with 50% oxygen saturation (P_{50} was determined from the regression line drawn through the points lying on the steepest portion of each curve. The P_{50} is a convenient, accepted way to express the relative position of the dissociation curve in relation to normality (27.5 mm. Hg O_2 tension).

For the *in vitro* studies, venous blood was obtained from normal adults and placed in tubes containing dry sodium heparin. Triiodothyronine (Cytomel), that had been solubilized by dissolving in dilute sodium hydroxide was added to a portion of each blood sample to a final concentration of 4.4 μg./ml. of whole blood. Four ml. blood samples were incubated in 25 ml. flasks for a period of 4 hours at 37° C. in a metabolic shaker oscillating at 80 rotations per minute. The samples were periodically gassed with a mixture of 5% CO_2 in room air and the pH was maintained between 7.37 and 7.44. Samples of whole blood containing no supplemental triiodothyronine were incubated in a similar fashion. At zero time and at 4 hours, samples were removed for 2, 3-DPG assays by the procedure previously cited.

Results

The correlation of the P_{50} with the level of red cell 2, 3-DPG, in the 33 patients is

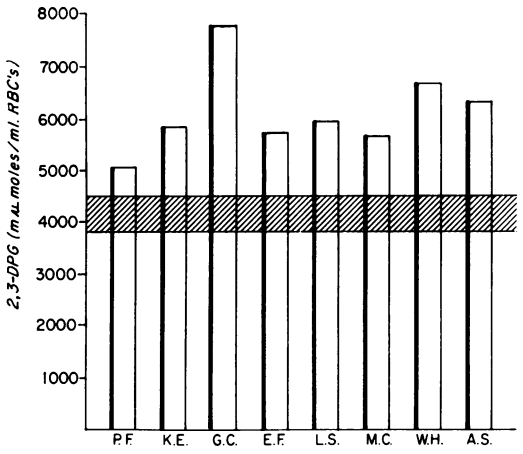

FIG. 2. Values for 2, 3-diphosphoglycerate (2, 3-DPG) in eight thyrotoxic patients, with normal values for 2, 3-DPG within the lined horizontal band.

very high (Fig. 1); the correlation coefficient was 0.97 with a p $(t) < 0.001$. The regression value was such that for every change, in either direction, of 420 millimicromoles of 2, 3-DPG there was a change of 1 mm. Hg in the P_{50}. The relationship is so precise, in fact, that one can accurately predict the position of the P_{50} from the 2, 3-DPG value alone.

Figure 2 illustrates individual values for 2, 3-DPG in eight patients with thyrotoxicosis before treatment was begun. Normal values for 2, 3-DPG, in almost 100 normal non-smokers has been found to be 4,200 ± 400 millimicromoles/ml. red cells. The average value in the eight hyperthyroid individuals is 6,120 mumoles/ml. red cells, which is 68.8% greater than control values.

Two patients were followed after treatment (Figs. 3 and 4). Patient L. S. (Fig. 3) received radioactive iodine (5.2 millicuries ^{131}I), as the primary treatment. She demonstrates the usual pattern, in that the progressive drop in 2, 3-DPG levels after therapy is apparent. Patient G. C. shows some variants from the standard pattern that are worthy of explanation. She received antithyroid medication methylmercaptoimidazole (Tapazole) until she became clinically euthyroid, and her basal metabolic rate was normal, and then underwent subtotal thyroidectomy (Fig. 4). Within 1 month with the antithyroid drug, she became euthyroid with a drop in her 2, 3-DPG from 7,734 to 4,806 mumoles/ml. red cells. Note, however, that for 2 weeks following subtotal thyroidectomy, her 2, 3-DPG levels returned to somewhat high values, although at this time she was clearly not toxic. These values may reflect an element of respiratory distress or anemia that this patient developed in the postoperative period. A persistent hypoxemia secondary to atelectasis could easily have caused this elevation in 2, 3-DPG. Two months following operation, she was still clinically euthyroid but the 2, 3-DPG level had risen to 6,536 mumoles/ml. red cells. During and after the immediate postoperative period, she was experiencing severe menstrual irregularities, resulting in a drop in hemoglobin values from 14 Gm./100 ml. to 10 Gm./100 ml. in 1 month. After dilatation and curettage at this point, and replenishment of red cell volume, 2, 3-DPG level fell to appropriately normal values. It is probable that the elevation in 2, 3-DPG noted previously was secondary, therefore, to the acute onset of anemia. It is clear then that several factors may be operative in the same patient, which will tend to increase oxygen

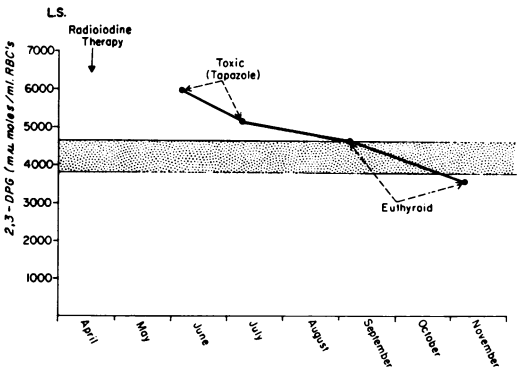

FIG. 3. Serial determinations of 2, 3-DPG in the course of returning to the euthyroid state, following radioiodine and Tapazole® therapy.

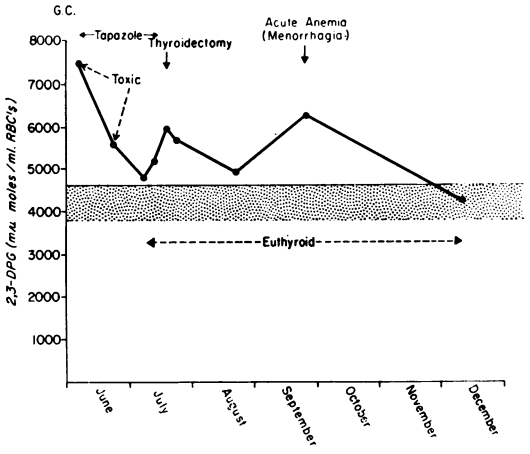

FIG. 4. Serial determinations of 2, 3-DPG in a patient prepared for surgery with Tapazole®. Complications of respiratory distress and acute anemia in the postoperative period.

demand and result in significant changes in 2, 3-DPG values.

One patient, A. S., illustrates one of the diagnostic advantages in following 2, 3-DPG levels in thyrotoxic patients. This patient was first seen at the Hospital of the University of Pennsylvania in November, 1968 with signs, symptoms, and standard tests of thyroid function, indicative of hyperthyroidism. She received an apparently adequate amount of radioactive iodine therapy at this time, since she became clinically euthyroid 6 to 8 weeks following treatment. In June, 1969, however, she again experienced nervousness, palpitations, sweating, and tremor. Nevertheless, ^{131}I uptake study revealed normal values (2 hours—9%; 24-hour—16%). Despite this finding, 2, 3-DPG level at this time was 6,320 mumoles/ml. red cells, well into the thyrotoxic range, without any other apparent reason for 2, 3-DPG elevation. The patient was then given 100 μc. of ^{131}I-tagged thyroxine and serial blood samples obtained for the next 7 days. Using the extrapolated O X ^{131}I-T_4 concentration, values were calculated for circulating extrathyroidal thyroxine. She was found to be degrading 113 μg. of thyroxine iodine per day or, approximately, twice the normal amount (normal = 53.6 ± 16.5 μg. per day). These kinetic values were consistent with hyperthyroidism. Therefore, the 2, 3-DPG levels, despite the normal ^{131}I uptake studies, gave a significant indication of the presence of an increased peripheral turnover of thyroid hormone. Without specific treatment, this patient underwent a spontaneous remission 3 to 4 months later, featured by the cessation of all previous signs and symptoms, and a drop in 2, 3-DPG to 3,899 mumoles/ml. Spontaneous remissions and exacerbations in this disease are common and may be followed accurately with serial 2, 3-DPG determinations.

In the normal volunteer (W. W. M.), receiving 200 μg. of triiodothyronine per day for 2 weeks, values of 2, 3-DPG, P_{50}, and oxygen consumption responded in similar fashion to those seen in the spontaneous disease. Levels of 2, 3-DPG rose from 4,375 to 5,760 mumoles/ml. (32%); the P_{50} changed appropriately from 26.0 to 30.5 mm. Hg; and oxygen consumption, originally determined at 126 ml./min./m.^{2}, increased to 146 ml./min./m.^{2} (16%).

Incubation of triiodothyronine, alone, with fresh blood, *in vitro*, produced a significant increment of 2, 3-DPG (Table 1). Whole blood relatively rapidly loses its 2, 3-DPG content if stored under normal blood bank conditions and, particularly, if left to stand at room temperatures. Therefore, each of the incubation experiments are contrasted with normal control depletion values. As calculated from the data in Table 1, the percentage increases in 2, 3-DPG, over control values, in four hour incubation studies are, respectively, 30.9%, 27.2%, and 46.6%. The exact site of action of the thyroid hormone on the Embden-Meyerhof cycle is, as yet, unknown. The incubation of red cells, *per se*, with triiodothyronine in buffer solution, does not produce the above results, suggesting that an inter-action between the hormone and a

constituent, or constituents, of plasma is necessary for the stimulation of 2, 3-DPG production.

Discussion

As stated, Figure 1 shows the close relationship between the 2, 3-DPG level and the value for the P_{50}. It can be seen that high levels of 2, 3-DPG, with resultant "shifts to the right" in the oxyhemoglobin equilibrium curve occur in a variety of conditions which necessitate an increase in oxygen supply, including thyrotoxicosis. The patients with "RBC" defects are individuals with inborn errors of red cell metabolism. The symbols in the normal range are for those with glucose-6-phosphate dehydrogenase deficiencies. Although these patients have episodic hemolytic episodes in response to several drugs, as well as infections and acidosis, the defect does not influence the production of 2, 3-DPG. However, the lowest value shown in Figure 1 and four of the highest values refer to an individual with hexokinase deficiency, and four children with pyruvate kinase deficiencies, respectively, which profoundly affect oxygen delivery, in opposite directions. The former enzyme block, fortunately rare, occurs prior to the steps resulting in the production of 2, 3-DPG in the Embden-Meyerhof glycolytic cycle and, therefore, results in precariously low levels of 2, 3-DPG, with marked "shifts to the left" in the P_{50}.[6] In contradistinction, pyruvate kinase-deficient individuals demonstrate some of the highest 2, 3-DPG values thus far recorded. Since the enzyme pyruvate kinase catalyzes the conversion of phosphoenolpyruvate to pyruvate, a block in the glycolytic pathway *beyond* the conversion steps to 2, 3-DPG, levels of the latter substance are allowed to progressively increase.[6] The enzyme deficiency states most clearly delineate, at both ends of the spectrum, the profound, controlling influence that 2, 3-DPG has on hemoglobin-oxygen equilibrium. A parallel delineation can be seen in the general physical state of the patients with these genetic defects. As one might anticipate, the hexokinase-deficient child and the pyruvate kinase-deficient child are quite different in their capacity for physical exertion. The "left-shifted" child cannot tolerate any physical exertion; the "right-shifted" child maintains full activity. Their ages and hemoglobin values are virtually identical. Patients with liver disease in Figure 1 represent several that we have studied with advanced cirrhosis; the patients with septic shock in the normal range were studied during the recovery phase, those "shifted to the left" were studied in the acute phase of their disease. "Shifts to the right," in addition to the types of patients presented in Figure 1, are also seen in chronic respiratory disease,[18] in adaptation to high altitude,[8, 17] and in chronic anemia.[7] Four patients with untreated thyrotoxicosis are also seen in Figure 1 and are, appropriately, "shifted to the right," with significantly high 2, 3-DPG levels.

It has been suggested that the neuro-endocrine interrelationships controlling thyroidal homeostasis are primarily directed toward bringing a metabolically adequate quantity of thyroid hormone to the pe-

TABLE 1. *Increased Production of Red Cell 2, 3-DPG When Incubated in Vitro with L-triiodothyronine*

2,3-DPG Before Incubation	μg Tri-iodothyronine /ml. RBC's	2,3-DPG After Incubation	% Change
4153	0	3480	−16.2
4153	4.4	4746	+14.7
4793	0	3895	−18.7
4793	4.4	5199	+8.5
3023	0	2420	−19.9
3023	4.4	3831	+26.7

In each study, the results are compared to the same amount of whole blood, allowed to stand, at room temperature, for the same time period (4 hours), without addition of the hormone.

ripheral tissues, regardless of the concentration at which delivery occurs.[14] Although measurement of serum protein-bound iodine is relatively simple and, usually, clinically useful, its concentration, at any one moment in time does not, necessarily, reflect the total turnover of thyroid hormones. The PBI is, in one sense, a composite of several vital, changing, interrelated factors in peripheral hormonal supply, such as thyroid hormone synthesis, peripheral uptake and degradation, and the amount of free hormone relative to the amount bound to the thyroxine-binding globulin or protein (TBG or TBP). In this regard, Ingbar[14] pointed out that a thyrotoxic patient who is turning over twice the amount of hormone, a hormone production rate that is four times normal will only result in a doubling of the PBI. In like manner, a significant increase in hormonal synthesis may result in a PBI which is increased only minimally, or not at all. An accelerated turnover of thyroxine has been observed in both untreated[4, 20] and in adequately treated Graves' disease,[15] as well as in thyrotoxicosis medicamentosa.[15] The finding of increased clearance in some patients with treated disease may logically explain the occurrence of a normal PBI in an individual who is clinically thyrotoxic, as well as others who have PBI's in the myxedematous range, while otherwise metabolically normal.[15] Although studies of labelled thyroxine turnover are being done currently at a number of centers, the determination is by no means simple. Moreover, as patient A. S. illustrates, ^{131}I uptake studies may also be normal in individuals with high hormonal turnover rates. The determination of 2, 3-DPG levels, therefore, seems to be a relatively accurate indicator of the amount of hormone actually being delivered to the peripheral tissues, in the absence of other clinical conditions characterized by increased oxygen demand, as enumerated above. Only three ml. of venous blood are required for the determination and even the small risk of isotopic studies, as in pregnant women, is precluded. Laboratories familiar with the technics of 2, 3-DPG determination may provide useful information in following the course of individuals before, during, and after treatment for hyperthyroidism.

The observation that the oxyhemoglobin equilibrium curve is "shifted to the right" in hyperthyroidism was first made in 1930, by Bansi and Groscurth.[1] Many years later, the observation was confirmed by several other investigators.[9, 10, 12] The primacy of 2, 3-DPG as the underlying etiologic mechanism in this "shift was recognized subsequently. It is now clear that a "shift to the right" is a normal compensatory mechanism, maintaining homeostasis in several clinical conditions in which relative oxygen lack is a prominent physiologic feature. It has been suggested by Valeri and Fortier that "an increase in 2, 3-DPG reflects an attempt by circulating red cells to compensate for any impairment of oxygen supply to tissue."[21]

Gahlenbeck and Bartels[10] showed that, in rats given triiodothyronine, a shift in the P_{50} of 4.1 mm. Hg supplied an additional increment of oxygen to tissues equivalent to 40% of the pre-existing oxygen consumption. Measured oxygen consumption in the volunteer in this study, secondary to self-administered T_3 for 2 weeks, increased 16%. There is no question that other adaptive mechanisms are operative in thyrotoxicosis, such as an increased cardiac output and a decreased systemic arteriolar resistance, all of which tend to increase peripheral flow and oxygen supply. The change in oxygen-hemoglobin equilibrium is a most appropriate additional attempt at adaptation, since oxygen supply to tissue may be significantly increased without any possibly disadvantageous stress on the myocardium. Figure 5 shows the projected advantage in terms of oxygen extraction, to

patient G. C., in whom a pre-treatment 2, 3-DPG elevation to 7,734 mumols/ml., with an appropriate shift in the equilibrium curve was recorded. For purposes of clarity, a single, normal P_{O_2} of 40 mm. Hg is chosen, but a proportionate increase in arterio-venous oxygen content difference would occur at any given P_{O_2} in the mid-range, with a right-shifted curve, as opposed to a curve in the normal position, as long as it is reasonably assumed that there is no significant decrease in arterial oxygen saturation concomitant with the Graves' disease state. These postulates do not detract, in any way, from the primary importance of providing respiratory and cardiac compensations for increased oxygen needs; they do, however, add one more significant facet to considerations of optimal provision for oxygen needs in this, as well as other, disease states.

The "physiologic signal" to the red cell to increase its oxygen delivery capability, by increasing 2, 3-DPG, is unknown. It has been presumed that some effect of decreased blood or of tissue oxygenation provides the stimulus. The T_3 incubation studies reported here (Table 1) suggests that the same, single "trigger mechanism" may not be operative in all clinical conditions in which there is an elevation of 2, 3-DPG. Although an increased oxygen demand is the common denominator in all these conditions, the effect of the thyroid hormones, *per se*, may influence red cell metabolism, and the cell's propensity to unload oxygen. In addition to increased circulating thyroid hormones, there appears, also, to be increased uptake of active hormone by the red cell. Hamolsky, and co-workers,[13] demonstrated that the uptake of ^{131}I-labelled l-triiodothyronine by red cells increases significantly in hyperthyroidism, without apparent correlation with PBI levels. The chemical concentration of triiodothyronine in the plasma has been determined in rela-

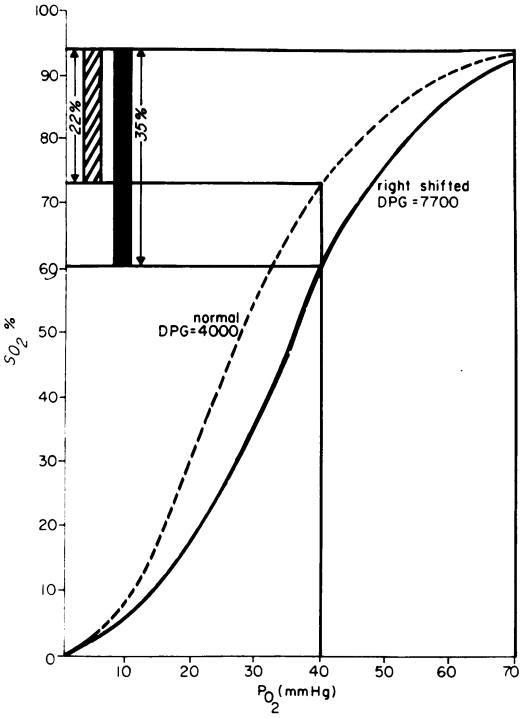

FIG. 5. Proposed advantage of the right-shifted oxyhemoglobin equilibrium curve in patient G. C., in terms of increased oxygen extraction, at a given venous P_{O_2}. Any increase in cardiac output would, of course, also contribute to an increased total oxygen consumption.

tively few patients, although following large doses of ^{131}I, appreciable quantities of this substance have been detected.[11] We must then approach the *in vitro* T_3 data with the reservation that the amount of triiodothyronine used may not prove to be in the physiologic range, even for the hyperthyroid individual. The fact that there is *any* increase in glycolytic intermediates within the red cell after short exposure to a naturally-occurring, metabolically active hormone appears to us to be of considerable significance. It is clear that the potential exists for high concentration of the thyroid hormones within the red cell, itself. This observation also raises the speculation that other polypeptide hormones may influence red cell, as well as other tissue, metabolism in a similarly appropriate way.

Summary

Levels of red cell 2, 3-DPG are elevated in Graves' disease, with resultant "shifts to the right" in the oxyhemoglobin equilibrium curves. Increases in this red cell glycolytic intermediate appeared to reflect peripheral elevations and increased turnover of metabolically active hormones. L-triiodothyronine alone may directly stimulate red cell production of 2, 3-DPG and, therefore, facilitate oxygen unloading by hemoglobin. Determinations of 2, 3-DPG may be used to follow, clinically, remissions and exacerbations in the Graves' disease state.

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