MAXIMAL HEMOGLOBIN AND PLASMA PROTEIN PRODUCTION UNDER THE STIMULUS OF DEPLETION*

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The maximal output of blood proteins in health is of considerable interest to students of protein metabolism. What is the ceiling for plasma protein production or hemoglobin output or both in the healthy dog? The purpose of the experiments here reported is to attempt to answer this question.

Past experiments (13, 3, 8) and present observations indicate that apparently 1 gm. per kilo per day is an approximation to the true value for maximal hemoglobin *or* plasma protein production in the healthy depleted dog receiving a large protein dietary intake. At this rate the depleted dog can replace all its circulating plasma proteins in 3 days and its circulating hemoglobin in 18 days—the ratio of plasma protein to hemoglobin in normal blood being about one to six. It can be stated with conviction that individual dogs vary as to their ceiling production of blood proteins and furthermore infection or intoxication may inhibit this favorable response to a rich protein diet with iron.

EXPERIMENTAL

The methods employed in the present experiments have been described in previous publications (10, 12).

The stimulus of anemia or hypoproteinemia is required to bring out maximal response in the production of hemoglobin and plasma protein. When we combine anemia and hypoproteinemia (Table 1) there is not complete summation and under these conditions the dog always produces about twice as much hemoglobin as plasma protein but the total protein output is again increased.

In some experiments where iron is given intravenously together with a favorable diet (Table 2) the output of hemoglobin and plasma protein may be pushed up to a ceiling of 1.5 gm. per kilo per day—in one experiment a weekly output 160 gm. total blood protein. This represents a return of 10 to 20 gm. new hemoglobin and plasma protein for every 100 gm. diet protein intake.

Anemia and hypoproteinemia can be produced simultaneously in the dog by judicious bleeding plus a diet low in protein containing 600 to 800 mg. iron and the necessary fat, carbohydrate, and accessories (9). These doubly

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depleted dogs maintain an anemia of 6 to 8 gm. per cent and a hypoproteinemia of 4 to 5 gm. per cent. The output of hemoglobin and plasma protein can then be controlled by the protein intake. These standardized dogs serve as valuable testing machines to evaluate amino acid mixtures (10), protein digests (10), and food proteins (9) as to their effect upon new blood protein production. These dogs are very susceptible to infections and intoxications as reported elsewhere (4, 6).

Table 1 shows 3 experiments with *combined anemia and hypoproteinemia* (double depletion). The protein diet intake was large and of excellent quality (liver and beef) plus iron (600 to 800 mg. per day). The output of hemoglobin and plasma protein was large and in some weeks attained a total of over 120 gm. The protein output was about 10 to 15 per cent of the intake which is about average for such a large protein intake which never is utilized as completely as are small amounts of diet protein under these conditions. There is a steady gain in weight and a strong nitrogen-positive balance which means that the body was forming and storing protein. Under these circumstances it is not possible to siphon off the equivalent of the protein built within the body in the form of blood proteins. With smaller protein intake we can draw off a mass of blood protein equivalent to the new protein formed—the dog being in weight and nitrogen balance. Under these circumstances the dog may return 20 to 40 gm. new blood protein for 100 gm. diet protein which means frugal and effective protein utilization.

Examination of the plasma protein levels in Table 1 shows clearly that with the large protein intake, the hypoproteinemia is not sustained below 5 gm. per cent. Bleeding is limited by hemoglobin production as we do not dare to reduce the hemoglobin level below 7 to 8 gm. per cent. This means that with this diet protein and iron intake the body reaches its maximal capacity for hemoglobin production but not for plasma protein production. Furthermore the positive nitrogen balance and weight gain indicate considerable *tissue protein accretion.* Our belief is that some of this body *protein accretion* (both new hemoglobin and tissue protein) goes through the *transition* or *mobile stage* of plasma protein—that the turnover of plasma protein is much larger than is measured by this plasma protein removal. Some considerable plasma protein (10) is removed within the body from the circulating pool to make new hemoglobin and supply tissue protein requirements in *addition* to that which we remove by bleeding. It can readily be shown that plasma protein as plasma given intravenously in the dog on a protein-free diet can effect much new hemoglobin production (10) and nitrogen equilibrium (5).

Hypoproteinemia alone may be produced by bleeding followed by a return of the red cells (plasmapheresis). The plasma protein level is then maintained at 4 gm. per cent by control of the protein intake and suitable plasmapheresis. It is difficult to measure *maximal plasma protein production* with

TABLE 1

Maximal Blood Protein Production—Grams per Week

Anemia and Hypoproteinemia

Period 1 wk.	Weight	Protein intake		Protein output weekly					Ratio protein output to intake	Total nitrogen gm.	
				Hemoglobin		Plasma Protein		Total output			
		Type	Weekly	Level	Output	Level	Output			Intake	Urinary output
	kg.		*gm.*	*gm. per cent*	*gm.*	*gm. per cent*	*gm.*	*gm.*	*per cent*	*gm.*	*gm.*
Dog 40-32											
1	16.6	Egg	128	6.0	1.1	4.8	0	1.1			
2	17.5	Liver, beef, iron	1037	8.1	64.5	5.8	40.5	105.0	10	166	35.9
3	18.7	" " "	1037	7.5	46.8	6.5	35.7	82.5	8	166	63.0
4	19.9	" " "	1037	8.5	46.6	6.3	30.5	77.1	7	166	73.6
5	20.4	" " "	1037	7.6	62.6	5.7	36.9	99.5	10	166	81.8
6	20.8	" " "	1037	9.8	27.2	6.1	16.9	44.1	4	166	75.8
7	21.7	" " "	1037	7.6	38.1	5.8	23.5	61.6	6	166	71.6
Average output per wk.					47.6		30.7	78.3			
8	20.9	Basal	19	7.7	85.1	5.2	47.1	132.2		3	22.0
9	19.9	"	19	7.1	30.0	4.3	17.4	47.4		3	11.7
10	19.2	"	19	7.1	1.6	4.2	0	1.6		3	
Dog 40-33											
1	18.5	Basal	19	8.6	26.3	3.9	9.0	35.3		3	9.3
2	18.6	Liver, beef, iron	973	7.8	89.0	5.1	44.9	133.9	14	157	37.4
3	18.8	" " "	1037	7.8	38.3	5.5	23.5	61.8	6	166	47.7
4	20.6	" " "	1037	9.5	69.4	5.4	38.6	108.0	10	166	57.0
5	20.2	" " "	1037	6.6	74.3	5.3	44.7	119.0	11	166	75.7
Average output per wk.					67.8		37.9	105.7			
6	20.7	Basal	19	6.6	23.3	4.6	10.7	34.0		3	18.3
7	18.3	"	19	10.3	17.9	3.8	6.6	24.5		3	10.1
Dog 37-85											
1	15.2	Basal	84	6.9	12.3	4.9	8.0	20.3		13	8.4
2	16.4	Liver, beef, iron	764	11.8	47.8	5.3	26.7	74.5	10	123	37.6
3	17.4	" " "	764	12.3	57.1	5.5	25.3	82.4	11	123	50.8
4	17.5	" " "	764	12.8	78.5	5.6	42.9	121.4	16	123	68.1
Average output per wk.					61.1		31.6	92.8			

a high protein diet to make new plasma protein. Even with a large bleeding and exchange twice daily the plasma protein level cannot be held at 4 gm. per cent and the absolute maximum capacity therefore cannot be measured. A number of such experiments have been completed (3, 8) and we may say in general that 1 gm. per kilo per day is close to the actual production ceiling.

Table 2 shows *maximal hemoglobin production under standard anemia conditions.* These dogs are kept continuously anemic at a hemoglobin level of 6 to 8 gm. per cent by suitable bleeding and are under this regimen for their complete adult life. Their response to various diet factors has been reported and reviewed from time to time (11). Liver is the best diet protein to effect

TABLE 2

Maximal Hemoglobin Production—Grams per Week

Standard Continuing Anemia of 6 to 8 Gm. Hemoglobin

Dog No.	Dog Average normal weight	Daily diet Liver 300 gm.	Daily diet Salmon bread plus iron 400–450 mg.			Daily diet Salmon bread, liver plus iron 400–450 mg.			Daily diet Liver, salmon bread plus iron by vein 24 mg.	
			Reduced	Ferric	Ferrous	Reduced	Ferric	Ferrous	Colloidal Fe	Estimated plasma protein removed
	kg.									
39-1	18.0	45*	60	51	50	62	58	66	92	71
40-26	14.5	50	59	45	60	56	63	—	80	49
37-21	18.0	41*	58	64	54	53	52	51	82	53
34-148	18.0	44*	49	49	54	47	63	71	106	58
34-145	20.0	47*	47	55	57	63	82	56	95	52
37-89	14.0	40*	32	54	42	39	48	47	84	47
33-14	12.0	38*	28	41	44	38	58	56	75	48
Average.......	16.3	44	48	51	52	51	61	58	88	54

* Average 2 to 6 experiments.

new hemoglobin production in anemia and Table 2 shows the average of many experiments to be 44 gm. per week with a diet intake of 300 gm. liver daily. In the same dogs iron salts (400 mg. daily) give a response of 50 gm. per week. Liver and iron combined do not give a summation but about 59 gm. per week (reduced iron excluded). When abundant food protein is given by mouth supplemented by intravenous iron we note a level of 88 gm. per week. This 50 per cent increase must be due to more available iron and we know that *iron absorption* is difficult. One *limiting factor* then is iron absorption. The body machinery for hemoglobin production can make more globin than the body can supply (through absorption) with iron. This observation emphasizes again the great capacity of the body to make new protein and channel the output into the area of acute need.

When we compare *hemoglobin production* in the anemic dogs (Table 2) with hemoglobin production in the anemic and hypoproteinemic dogs (Table 1), the figures are close but the doubly depleted dogs if anything produce a little more new hemoglobin per week. Perhaps the double stimulus drives the body to even greater productive efforts. There is no hypoproteinmeia in the anemic dogs and frequent sampling shows an average plasma protein level of about 6 gm. per cent. The output of new plasma protein during intravenous iron administration is considerable and is shown in the last column of Table 2. This amounts to an estimated 50 to 60 per cent of the removed hemoglobin.

In the last column of Table 2 are shown the approximate amounts of plasma protein removed in the anemia experiments using intravenous iron. These figures are *estimates* using the amounts of plasma and 6 gm. per cent as a uniform factor of protein concentration. Sampling of anemic dogs in periods of active hemoglobin regeneration shows an average of about 6 gm. per cent. The values given are certainly close to true values of analyses and the average figure of 54 gm. plasma protein per week is considerable. This figure exceeds the plasma protein figures in the doubly depleted dogs in Table 1 (31, 38, 32 gm. per week) but not the maximal figures obtained in the hypoproteinemic dog with plasmapheresis (60 to 70 gm. per week) cited above.

Iron absorption is a phrase much used and little understood—the medical literature is cluttered up with misinformation. As is usually the case these divergent statements are due to limited understanding of this phenomenon in animals and man. The use of *radioactive iron* has been of great help in solving some of the fundamental problems related to iron absorption, elimination, and turnover in the body (1, 2). It is becoming evident that the dog differs a little in the absorption of iron when compared with man.

Table 2 shows again that ferrous and ferric iron salts in the anemic dog are used with equal facility. Reduced iron falls a little behind. The body can use more iron in these experiments than it can absorb, as is readily seen when iron is given by *vein,* effecting a 50 per cent increase in hemoglobin output. In the human anemic patient the ferrous iron is more readily absorbed in most cases (7).

SUMMARY

The maximal output ceiling for hemoglobin in anemia due to blood loss is about 60 gm. per week—the dog receiving a rich protein diet plus high iron intake. Ferrous and ferric salts are equally effective. Iron intravenously plus a rich protein diet may push this level up to 90 to 100 gm. per week. Evidently iron absorption is a limiting factor.

Maximal output for hemoglobin plus plasma protein in doubly depleted dogs may reach 120 to 130 gm. per week and using intravenous iron may reach 140 to 160 gm. per week.

Maximal output for plasma protein alone in hypoproteinemia due to plasma-

pheresis reaches 60 to 70 gm. per week but *this is not the true ceiling*. Technically we cannot remove the new plasma protein as fast as it is formed and the hypoproteinemia is not maintained in the face of a rich protein diet intake. Furthermore the evidence points to the protein circulating pool contributing to the accretion of tissue protein in such dogs with a strong positive nitrogen balance and weight gain.

Maximal figures for *hemoglobin production* in anemia run close to 1 gm. hemoglobin per kilo per day. Maximal figures for new *hemoglobin plus plasma protein* production in anemia and hypoproteinemia using iron given intravenously, may reach 1.5 gm. blood protein per kilo per day. The actual maximal *plasma protein production* equals about 1 gm. per kilo per day but the true production ceiling cannot be reached by this technique, for reasons given above.

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