groups is given in Table VI. This shows that whereas in the Aberdeen series not a single child had a haemoglobin of less than 80%, almost half of the children in Helen Mackay's series and more than half of the children examined by us in Edinburgh had haemoglobin concentrations below that level.

TABLE VI.—Comparison of Anaemia in 3 Groups of School Children (5 to 12 Years)

	No. of Cases	Percentage of Children in Different Haemoglobin Ranges			
		Hb < 71%	Hb 71-80%	Hb 81-90%	Hb > 90%
Edinburgh, 1942	389	8	45	40	7
London (Mackay, 1942)	128	53		47	
Aberdeen (Davidson, 1935)	67	42		68	
		0		100	

The children and also the infants in the Aberdeen investigation were drawn from the poorest section of the community at a time when the unemployment rate was extremely high. The weekly income assessed on the basis of "man value" averaged only about 11s. per head in Aberdeen, in contrast with the present Edinburgh assessment of the family income of 520 women examined at ante-natal clinics, which ranged from 24s. to 39s. per "man value" per week.

It will be appreciated that in peacetime economic status is the chief factor influencing the nutrition of individuals; in wartime, on the contrary, "rationing" and the difficulty in obtaining some unrationed foods constitute the chief limitations, which, broadly speaking, are operative irrespective of income. This is probably the explanation of the higher incidence of anaemia noted by us during the present year in spite of the high figures for "man value" recorded. In support of this conclusion it is significant to note that since obtaining the data already recorded we have examined the haemoglobin concentration of 134 school children aged 5 to 12 years who were pupils in an Edinburgh secondary fee-paying school and who were drawn from a higher social and economic class than the children considered above. The average haemoglobin level for this group was 80.7%, a figure almost identical with that obtained for the 389 working-class children. The percentage of children falling in the different haemoglobin ranges was also similar.

The frequency and degree of anaemia present in school children from working-class families in Edinburgh in 1942 as reported in this paper, call for the most careful consideration by both local and national authorities. It is obviously desirable that during the period of active growth and mental development the incidence of anaemia should be reduced to the lowest possible level. This can be achieved by one of two methods or by a combination of both. The first consists in the provision of school meals which are appetizing, well balanced, and rich in foodstuffs with a high iron content. Intensive propaganda must be undertaken to induce a much higher proportion of children to partake of such meals. The need for such propaganda is evident in Edinburgh where, although the numbers are gradually rising, only some 20% of children are at present taking school meals. The second method is by supplying iron at the schools in the form of a tablet or a mixture, or incorporating it in some article of diet universally eaten, such as bread.

Investigations are at present being undertaken in Edinburgh to discover whether the anaemia can be prevented and corrected by diet alone or whether a supplement of iron is required.

Summary

The haemoglobin levels have been estimated in 831 infants, pre-school children, and school children from working-class families in Edinburgh. The results have been compared with a similar series examined in Aberdeen in 1935.

After three years of war no significant change was found to have occurred in the haemoglobin levels of infants, but a fall has been demonstrated in the case of school children.

* The "man value" figures obtained by us were calculated on the following basis: each member of the family was assessed according to age and sex in terms of one man as one unit, one woman as 0.8 of a man, and a child as 0.5 of a man.

We wish to express our thanks to Prof. Charles McNeil, Dr. G. J. Linklater, school medical officer, and Dr. T. Y. Finlay, medical officer for maternity and child welfare in Edinburgh, for the facilities and help afforded us by them during this investigation.

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PLASMA PROTEIN STORAGE

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In a previous paper (Beattie and Collard, 1942) it was stated that after a moderate haemorrhage there was a significant movement of plasma protein into the blood stream. This conclusion was reached by demonstrating that the observed plasma protein concentration of the blood after haemorrhage was greater than a value calculated from the haemoglobin concentration before and after haemorrhage. Dr. Sharpey-Schafer has pointed out to us in a personal communication that the formula used was incorrect and gave expected values which were too high. He suggested the following formula:

$$p_1 = p \times \frac{1}{1 + \frac{1}{x} \left(\frac{H}{H_1} - 1 \right)}$$

Where p_1 is the plasma protein concentration to be expected at, say, 75 minutes after haemorrhage, p is that observed at, say, 30 minutes after haemorrhage, H and H_1 the haemoglobin concentration at 30 and 75 minutes after haemorrhage, and x is the plasma volume % divided by 100 at 30 minutes after haemorrhage. Using this method of calculating the expected value for the plasma protein concentration, we found that we had overestimated the true expected value by varying amounts ranging from about 3 to 15%. Consequently the movement of protein into the blood stream after haemorrhage was, in fact, much greater than we had reported. We are grateful to Dr. Sharpey-Schafer for pointing out our error. This movement of protein observed in anaesthetized cats has been recorded in human subjects bled comparable amounts (Stead, 1940; Wallace and Sharpey-Schafer, 1941). The view was expressed by us that the amount of protein entering the blood was determined probably more by the state of the protein reserves than by any other factor. After plasma transfusions in normal animals there is some evidence of an indirect nature that a movement of plasma protein out of the blood stream may take place (Beattie, 1942). As this outward movement was not constant in all experiments it may well be that it, too, is determined by the state of the protein reserves.

The sites of these reserve stores of protein have not been determined with certainty, but Madden and Whipple (1940), in a review of the evidence, considered that the liver was probably the most important store, although they admitted that direct evidence from hepatectomized animals and perfused liver experiments is at best equivocal. It was decided, therefore, to study the effects of plasma transfusions on hepatectomized animals and of varying protein concentrations in the perfusing fluid in perfused isolated livers. In addition, it was thought that by giving repeated plasma transfusions to normal animals it might be possible to determine whether the plasma protein reserves could be filled up rapidly and so allow the building up of high plasma protein concentrations in the blood within a relatively short time.

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Methods

The animals used in all the experiments were cats anaesthetized with nembutal (50 mg. per kg. of body weight). They were heparinized during the experiments. Plasma used for transfusions, etc., was obtained from heparinized cats. The cells were separated immediately after the blood was withdrawn and the separated plasma stored at a temperature of 6° C.

Chemical.—Plasma proteins were estimated by the biuret method of Robinson and Hogden (1940) and haemoglobin by the alkaline haematin method of Wu (1922). Plasma volumes were determined by the dye method using T.1824 (Evans blue). Dye concentrations were estimated by a photo-electric colorimeter. A regression curve plotted from the dye concentrations in samples taken at 10-minute intervals was used to obtain the plasma volume before dye was injected (Hamlin and Gregersen, 1939). Deviations from the regression curve were used to estimate variations in plasma volume as described by the same authors. Fresh injections of dye at hourly intervals were given to check the values calculated from the regression curve.

Plasma specific gravities were estimated by the falling-drop method of Barbour and Hamilton (1926), and from these the plasma protein concentration was estimated by the formula suggested by Weech *et al.* (1936). These estimations were of value only in normal and hepatectomized animals, in which they checked closely with the values obtained by the biuret method. Plasma S.G. estimations are valueless in perfused liver experiments, as they show continued rises during the perfusions irrespective of changes in protein content (as determined by the direct biuret method). The substances or substance which gradually increased in concentration in the perfusing fluid were not determined, but were probably urea, lactate, or glucose, or a mixture of all. The pH of the perfusing fluid was not determined.

Physiological.—The livers for perfusion were removed after the animals had been bled rapidly from the abdominal aorta. An inflow cannula was tied in the inferior vena cava above the diaphragm. The common bile duct was ligated and a cannula tied into the gall-bladder in all except two animals which had small gall-bladders. The phrenic vessels were ligated close to the inferior vena cava, and the liver was then removed. It was placed on a cotton gauze sling, with the portal vein and hepatic artery free to drain through the gauze into a porcelain funnel. This was covered with a perspex lid through which projected the two cannulae. Perfusion was carried out in reverse, the inflow being through the inferior vena cava and the outflow through the portal vein. The perfusion pressure was maintained constant at 12 to 15 c.cm. above the upper surface of

the liver by a reservoir fed by a pump which raised the perfusion fluid from the oxygenator. The outflow from the liver was passed into the oxygenator, where it fell on to a rotating perspex disk which threw the fluid on to the walls of a glass cylinder through which a stream of oxygen passed. The fluid collected at the bottom of the oxygenator, and from there was raised to the reservoir by a pump. The porcelain funnel and the oxygenator were immersed in water baths maintained at a constant temperature of 40° C. The temperature of the perfusing fluid was 36° to 38° C.

Hepatectomized animals were prepared by the one-stage method of Firor and Stinson (1929).

Observations

Repeated Plasma Transfusions.—Five animals (91, 92, 93, 100, and 106) were given repeated plasma transfusions. The results of these transfusions on the plasma protein concentrations are shown in Tables I to III. No. 91 was given 4 transfusions, each of 10 c.cm., at intervals of 30 minutes. In spite of the fact that the animal's plasma contained 8.3 g. per 100 c.cm., the transfusion of the relatively low protein plasma (6.84 g. per 100 c.cm.) caused a transient rise in the plasma proteins (8.64 g.), followed by a decline which brought the plasma proteins (8.06 g.) below the pre-transfusion level. A second transfusion of the same plasma produced no significant change in the protein concentration at the end of 29 minutes. A third transfusion of the same plasma produced a significant rise to 8.40 g. per 100 c.cm. in 29 minutes. The fourth transfusion, again of the same plasma, raised the protein level to 8.84 g. at the end of 45 minutes, and it remained at that level at the end of 60 minutes.

The changes seen in No. 92 resemble closely those in No. 91. This animal had an initial plasma protein concentration of 6.67 g. per 100 c.cm. It received 3 transfusions, each of 20 c.cm., at 45-minute intervals. The plasma transfused had a protein content of 6.05 g. per 100 c.cm. The first transfusion caused a transient rise in protein concentration to 7.24 g. in 16 minutes. The value had declined to 6.94 g. at the end of 41 minutes. The second transfusion produced a considerable rise to 7.72 g. in 10 minutes, which was maintained until the third transfusion was given. This caused a further rise to 7.99 g., and this level was maintained with some minor fluctuations for 55 minutes.

No. 93 was given 3 transfusions, the second 42 minutes after the first, and the third 118 minutes after the second. The first 2 transfusions were of 15 c.cm. and the third was 10 c.cm. The protein concentration of the transfused plasma was 7.14 g. per 100 c.cm. After a fall, the pre-transfusion level was almost regained 41 minutes after transfusion. The second transfusion produced no change in protein concentration, but the third caused a well-marked

TABLE I

No.	Plasma Volume before 1st Transfusion (c.cm.)	Plasma Protein Concentration before 1st Transfusion	Volume of Plasma Transfused (c.cm.)	Plasma Protein Concentration of Plasma Transfused	Plasma Protein Concentrations after 1st Transfusion (grammes per 100 c.cm.)							
					Min. After	Pl. Prot. Conc.	Min. After	Pl. Prot. Conc.	Min. After	Pl. Prot. Conc.	Min. After	Pl. Prot. Conc.
91	87	8.30	10	6.84	9	8.64	14	8.40	28	8.06	—	—
92	195	6.67	20	6.05	10	7.21	16	7.24	26	7.14	41	6.94
93	104	8.57	15	7.14	10	8.40	16	8.09	26	8.13	41	8.50
100	151	7.69	20	6.26	—	—	25	8.23	40	8.16	55	8.13
106	124	7.28	20	6.46	10	7.58	25	7.07	40	7.18	55	7.11

TABLE II

No.	Time between 1st and 2nd Transfusions (min.)	Plasma Protein Concentration before 2nd Transfusion	Volume of Plasma Transfused (c.cm.)	Plasma Protein Concentration of Plasma Transfused	Plasma Protein Concentrations after 2nd Transfusion (grammes per 100 c.cm.)							
					Min. After	Pl. Prot. Conc.	Min. After	Pl. Prot. Conc.	Min. After	Pl. Prot. Conc.	Min. After	Pl. Prot. Conc.
91	30	8.06	10	6.84	10	8.20	15	8.09	29	8.09	—	—
92	42	6.94	20	6.05	10	7.72	16	7.52	26	7.75	41	7.62
93	42	8.50	15	7.14	10	8.60	15	8.57	26	8.54	45	8.57
100	60	8.13	20	6.36	11	8.09	25	8.13	40	8.16	—	—
106	60	7.11	20	6.46	10	7.86	25	7.96	40	8.03	—	—

TABLE III

No.	Time between 2nd and 3rd Transfusions (min.)	Plasma Protein Concentration before 3rd Transfusion	Volume of Plasma Transfused (c.cm.)	Plasma Protein Concentration of Plasma Transfused	Plasma Protein Concentrations after 3rd Transfusion (grammes per 100 c.cm.)							
					Min. After	Pl. Prot. Conc.	Min. After	Pl. Prot. Conc.	Min. After	Pl. Prot. Conc.	Min. After	Pl. Prot. Conc.
91	30	8.09	10	6.84	10	8.26	15	8.26	29	8.40	—	—
92	43	7.62	20	6.05	10	7.99	15	7.75	25	7.92	40	7.89
93	118	8.50	10	7.14	10	8.67	15	9.22	25	9.15	40	9.25
100	45	8.16	20	6.36	10	8.13	25	8.13	40	8.16	55	8.30
106	45	8.03	20	5.88	10	8.26	25	8.26	40	8.33	—	—
*91	30	8.40	10	6.84	10	8.81	15	8.67	30	8.74	45	8.84

* These values were obtained for a 4th transfusion.

TABLE IV

No.	Composition of Perfusing Fluid	Plasma Protein Concentrations in Grammes per 100 c.cm.										
		0 Min.	15 Min.	30 Min.	45 Min.	60 Min.	75 Min.	90 Min.	105 Min.	120 Min.	135 Min.	150 Min.
150	{ 50 c.cm. plasma 180 c.cm. Tyrode's soln. } ..	1.63	—	1.69	—	1.71	—	*1.75	1.47	1.50	1.52	1.55
153	{ 80 c.cm. red cells 170 c.cm. plasma } ..	6.23	6.23	6.32	6.34	6.30	6.15	6.25	6.23	—	—	—
154	{ 130 c.cm. plasma 40 c.cm. Tyrode's soln. } ..	3.56	3.50	3.53	3.38	3.42	3.47	3.53	3.62	3.68	—	—
172	{ 95 c.cm. plasma 55 c.cm. blood } ..	6.49	6.32	6.37	6.34	6.28	6.11	6.27	6.24	—	—	—
173	{ 65 c.cm. plasma 100 c.cm. blood } ..	6.82	6.71	6.76	6.65	6.50	6.63	6.61	6.45	6.49	—	—

* 50 c.cm. Tyrode's solution added after this sample.

rise to 9.22 g. in 15 minutes. This new level was maintained for a further 70 minutes.

In No. 100 the second transfusion was given 1 hour after the first, and the third 45 minutes after the second. The plasma transfused was, as in the previous experiments, lower in protein content than the animal's own plasma. The first transfusion caused a rise in protein content from 7.69 to 8.23 g. per 100 c.cm. in 25 minutes. There was a slight decline over the remainder of the first hour. The second transfusion produced no change in protein concentration, and the third likewise, except at the final sample taken 55 minutes after this transfusion, when the protein concentration rose slightly to 8.3 g.

The reaction to the first transfusion in No. 106 was almost identical with that observed in No. 91. The second transfusion, 1 hour later, produced a considerable rise in protein content (7.11 g. to 7.86 g. per 100 c.cm.) in 10 minutes. The protein content continued to rise slightly for a further 30 minutes. The third transfusion, 45 minutes after the second, again caused a rise (8.03 g. to 8.26 g.), which was maintained for 40 minutes. The plasma used for the first and second transfusions had a protein content of 6.46 g. per 100 c.cm., while that for the third transfusion contained only 5.88 g. The protein content of the animal's plasma was considerably in excess of that in the transfused plasma.

Perfused Isolated Livers.—These experiments fall into two groups: (i) 5 experiments in which no plasma was added after the start of the experiment; and (ii) 4 experiments in which plasma was added. The results obtained in the first group are shown in Tables IV and V. The changes in plasma protein concentration are given in Table IV. Table V is calculated after allowing for losses due to

TABLE V

No.	Total Plasma Protein in Circulation at Start of Experiment A	Total Plasma Protein Lost by Sampling B	Expected Total Plasma Protein at End of Experiment C	Actual Total Plasma Protein at End of Experiment D	Difference between Cols. C & D as % of Expected Protein
150	3.75	0.32	3.43	4.03	+17.5
153	10.59	1.25	9.34	9.35	—
154	6.05	0.32	5.73	5.93	+3.5
172	8.11	0.79	7.32	7.02	-4.1
173	8.53	0.60	7.93	7.24	-8.7

sampling. In the 2 livers (Nos. 150 and 154) perfused with low protein perfusing fluid there was a rise in plasma proteins. The increase is greatest in the liver perfused with the lowest plasma protein fluid (No. 150). No change was found in No. 153, which was perfused with a fluid containing plasma protein in the concentration of 6.23 g. per 100 c.cm. Livers perfused with fluid containing a higher plasma protein concentration than 6.23 g. per 100 c.cm. (Nos. 172 and 173) apparently removed protein from the circulation.

TABLE VI

No.	Composition of Perfusing Fluid	Vol. of Plasma Added (c.cm.)	Pl. Prot. Conc. of Added Plasma	Protein in Added Plasma (g.)	Plasma Protein Concentrations in Grammes per 100 c.cm. at 10-Minute Intervals												
					0	10	20	30	40	50	60	70	80	90	100	110	120
164	200 c.cm. plasma-Tyrode mixture	20	6.53	1.31	2.70	2.80	2.95	2.99	—	2.99	*3.05	3.19	—	3.40	3.49	3.46	—
165	242 c.cm. plasma-Tyrode mixture	19	5.79	1.10	2.53	2.53	2.66	2.45	2.53	*2.53	2.62	2.89	2.88	2.59	2.66	2.57	2.51
167	170 c.cm. plasma, 38 c.cm. normal saline	20	6.13	1.23	6.08	5.86	5.84	5.73	5.83	*5.58	5.83	5.89	5.93	*6.08	5.10	5.03	5.30
170	125 c.cm. plasma	25	7.40	1.85	5.47	5.36	5.48	5.05	5.08	5.46	*5.67	5.47	5.72	5.77	5.74	5.89	5.89

* Plasma added after this sample. † Tyrode's solution added after this sample.

The second group again had experiments with low and high protein content in the perfusing fluid (Tables VI and VII). No. 164, perfused with a plasma containing only 2.7 g. per 100 c.cm., showed an increase in protein concentration over a period of 60 minutes of 13%. The percentage increase in concentration in the second hour was 13.4, although 1.31 g. of protein had been added. Over the

whole 2 hours there was an increase in total plasma protein of 7.9%. No. 165, also perfused with fluid of low protein content, showed no significant change in concentration over a period of 50 minutes. When 1.10 g. of protein was added the concentration returned to its initial level about 1 hour later. Protein had been lost from the perfusing fluid. Perfusion with a much higher protein concentration in No. 167 led to a fall of 8.2% in concentration in

TABLE VII

No.	Total Plasma Protein in Circulation at Start of Experiment A	Total Plasma Protein Added to Circulation B	Total Plasma Protein Lost by Sampling C	Expected Total Plasma Protein at End of Experiment (A+B-C) D	Actual Total Plasma Protein at End of Experiment E	Difference between Cols. D & E as % of Expected Protein
164	5.40	1.31	0.32	6.39	7.01	+9.7
165	6.12	1.10	0.34	6.88	6.22	-9.6
167	12.65	1.23	0.90	12.98	12.95	—
170	6.56	1.85	0.72	7.69	7.89	+2.6

50 minutes. After 1.23 g. of protein had been added, the concentration returned to the starting level. The addition of 40 c.cm. of Tyrode's solution failed to produce any output of protein from the liver, and consequently the total protein at the end of the experiment was almost identical with that expected. The last experiment (No. 170) showed a rise in plasma protein concentration of 3.7% in 1 hour. The addition of 1.85 g. of protein increased the concentration by a further 3.9%. The gross increase in total plasma protein, however, exceeded by only 2.6% the value expected.

Hepatectomized Animals.—These animals were observed for a control period before giving plasma transfusions. The changes in their plasma protein and haemoglobin concentrations are shown in Table VIII. Of these, 4 animals (Nos. 147, 158, 162, and 168) maintained their haemoglobin concentrations within $\pm 3\%$ of the first value. An examination of the dye disappearance curve shows that their plasma volumes appeared to be constant within narrow limits. No observations of plasma volumes were made on No. 168. No. 161 showed a marked increase in plasma volume and a corresponding reduction in haemoglobin concentration. The plasma protein concentration did not fall parallel with the fall in haemoglobin concentration. No. 166 showed an actual increase in plasma protein concentration, with a reduction in haemoglobin concentration and a rise in plasma volume. Calculating the changes in total plasma proteins, it was noted that Nos. 147 and 158 showed no evidence of movement of plasma protein into or out of the blood. No. 162 showed a decline of 4%, which is probably significant. Nos. 161 and 166 showed evidence of movement of plasma proteins into the blood (Table IX).

After a known amount of plasma had been transfused into 4 animals the changes in haemoglobin and plasma protein concentrations were observed and plasma volume estimations made (Table X).

An analysis of these results (Table XI) showed that 3 out of the 4 animals (Nos. 147, 158, and 162) lost protein in substantial amounts, and 1 animal (No. 161) gained over 5% plasma protein. Without placing too much reliance on the actual values, it is clear that plasma protein movement out of and into the blood stream does take place in hepatectomized animals.

Discussion

After repeated transfusions with plasma of a protein content lower than that of the animal's blood, the plasma protein content showed significant rises in 4 out of the 5 animals, and a rise of doubtful significance in 1 (No. 92). Analysing these results, it can be seen from Table XII that 4 animals (Nos. 91, 92, 93, and 106) lost the major portion of the transfused fluid from the circulation. One animal (No. 100) lost about half the transfused fluid.

The plasma protein added to the blood was retained in the circulation in 2 experiments (Nos. 92 and 100), and considerable fractions of it left the circulation in the others (Nos. 91, 93, and 106). Presumably, the greater part of this "lost" protein entered the protein stores.

Retention of plasma protein in the circulation may be accomplished by increasing the concentration to a much higher level and by expanding the blood volume. In No. 92 the former factor is predominant, and in No. 100 the latter. The question may be asked: "What determines whether plasma protein moves from the blood or is retained in it?" It has been suggested (Beattie and Collard, 1942) that the state of the plasma protein stores may be the most important factor.

If this be the explanation, then we have to assume that the plasma stores in Nos. 92 and 100 were well filled and could accommodate no more plasma protein. Examining these experiments in some detail, the effects of the first transfusion of low protein plasma are of interest. Determining the total plasma proteins from the animal's and transfused plasma volumes and the plasma protein concentrations, this value is divided by the concentration observed at certain time-intervals, thus giving the plasma volume to be expected. Assuming that no plasma protein has entered the blood stream, the plasma volume of No. 92 at 10 minutes after transfusion ought to be 197 c.cm., which would mean that 18 c.cm. of fluid had disappeared from the blood. However, as the haemoglobin concentration had declined from 102.0 to 95.84, it is clear that such a movement of fluid could not have occurred. Plasma protein must therefore have left the plasma stores and entered the blood stream. The subsequent fall in plasma protein may be due either to movement of fluid into the blood or protein out of it. If the former were the cause, the haemoglobin concentration would show a continued fall; in fact, however, it rose slightly to 97.54 at 16 minutes and 97.84 at 26 minutes, and fell slightly to 97.0 at 41 minutes. It must be concluded that there has been a reverse movement of protein into the stores.

TABLE VIII.—*Hepatectomized Animals*

Haemoglobin and Plasma Protein Concentrations before Transfusion

No.	Time (min.)	Hb	P.P.	Time (min.)	Hb	P.P.	Time (min.)	Hb	P.P.	Time (min.)	Hb	P.P.	Time (min.)	Hb	P.P.	Time (min.)	Hb	P.P.
*147 ..	0	100.0	5.65	11	103.0	5.61	21	101.6	5.65	31	102.0	5.51	—	—	—	—	—	—
158 ..	0	100.0	7.35	11	98.63	7.21	21	100.3	7.18	31	100.0	7.18	41	100.0	7.14	—	—	—
161 ..	0	100.0	8.33	15	94.4	8.03	20	91.8	—	25	91.8	7.86	30	92.1	7.96	—	—	—
*162 ..	0	100.0	7.00	10	100.3	6.66	20	100.3	6.66	30	103.0	—	40	103.0	6.86	—	—	—
166 ..	0	100.0	6.58	15	96.8	6.70	25	97.4	6.67	35	95.0	6.73	45	94.4	6.82	—	—	—
168 ..	0	100.0	7.11	12	99.3	7.18	24	99.65	7.18	34	98.6	7.07	44	98.95	6.87	53	98.3	6.60

* These animals received either plasma or glucose (5%) before this sample.

TABLE IX

No.	Beginning of Experiment			Duration of Experiment (min.)	End of Experiment					Gain or Loss as % of Total P.P. at Beginning of Experiment
	P. Vol.	P.P. Conc.	Total P.P.		P. Vol.	P.P. Conc.	P.P. in Circulation	P.P. Lost in Sampling	Total P.P.	
147	70	5.65	3.96	31	69	5.51	3.80	0.17	3.97	—
158	68	7.35	5.00	41	68	7.14	4.86	0.14	5.00	—
161	133	8.33	11.08	30	144	7.96	11.46	0.16	11.62	+4.9
162	101	7.00	7.07	40	97	6.86	6.65	0.14	6.79	-4.0
166	124	6.58	8.16	45	131	6.82	8.93	0.13	9.06	+11.0
168	—	7.11	—	53	—	6.60	—	—	—	—

TABLE X.—*Hepatectomized Animals*

No.	P.V. before Trans. (c.cm.)	Vol. of P. Trans. (c.cm.)	P.P. Added (g.)	Hb and P.P. Conc. after Plasma Transfusion. 10-minute Intervals											
				0		10		20		30		40		50	
				Hb	P.P. Conc.	Hb	P.P. Conc.	Hb	P.P. Conc.	Hb	P.P. Conc.	Hb	P.P. Conc.	Hb	P.P. Conc.
147	69	25	1.78	102.0	5.51	89.8	6.22	87.9	6.19	84.9	5.92	82.7	5.82	82.0	5.68
158	68	30	1.92	100.0	7.14	80.4	7.18	78.2	7.11	77.7	7.01	80.4	6.94	—	—
161	144	45	2.64	92.1	7.96	74.1	7.86	74.4	7.65	75.4	7.86	75.2	7.79	73.1	7.79
162	97	40	1.20	103.0	6.86	90.0	6.19	85.6	6.37	88.2	6.14	89.1	6.23	89.4	6.19

TABLE XI

No.	Beginning of Experiment			P.P. Added by P. Trans. (g.)	Total P.P. at End of P. Trans. (g.)	End of Experiment					Gain or Loss as % of Total P.P. at Beginning of Experiment
	Vol.	P.P. Conc.	Total P.P.			P. Vol.	P.P. Conc.	P.P. in Circulation	P.P. Lost in Sampling	Total P.P.	
147	69	5.51	3.80	1.78	5.58	85	5.65	4.80	0.41	5.21	-6.6
158	68	7.14	4.86	1.92	6.78	85	6.94	5.90	0.14	6.04	-10.9
161	144	7.96	11.46	2.64	14.10	182	8.03	14.61	0.23	14.84	+5.3
162	97	6.86	6.65	1.20	7.85	103	6.02	6.20	0.19	6.39	-18.6

TABLE XII

No.	Before Transfusion			Vol. of Plasma Trans. (c.cm.)	P.P. Added (g.)	End of Experiment			Net Gain or Loss of P.P. (g.)	Net Gain or Loss of Fluid (c.cm.)
	P.V. (c.cm.)	P.P. Conc.	Total P.P. (g.)			P.V. (c.cm.)	P.P. Conc.	Total P.P. (g.)		
91	87	8.30	7.22	40	2.74	93	8.84	8.22	-1.74	-34
92	195	6.67	13.01	60	3.63	209	8.09	16.90	+0.26	-46
93	104	8.57	8.91	40	2.86	107	9.28	9.93	-1.84	-47
100	151	7.69	11.61	60	3.80	184	8.30	15.27	-0.14	-27
106	124	7.28	9.03	60	3.76	140	8.33	11.66	-1.13	-44

The second transfusion produced a similar effect. The plasma protein concentration rose sharply at 10 minutes and, after fluctuating at 16 and 26 minutes, fell slightly at 41 minutes. The haemoglobin concentration moved in the reverse direction, rising from 97.24 at 10 minutes to 100.1 at 41 minutes after transfusion. Flow and ebb of plasma protein from and into the stores must have occurred. The third transfusion showed clearly that the same events have taken place. As the haemoglobin 10 minutes after transfusion was identical with that at 55 minutes after, no substantial movement of fluid could have occurred. Hence the final rise in plasma protein concentration must have been due to the plasma protein added from the stores.

An analysis of No. 100 showed that the large loss of transfused fluid was shared equally between the three transfusions. The loss was rapid and complete within 10 minutes, as the haemoglobin concentrations at the 10-minute and subsequent samples after each transfusion were within 1.6% of each other. The changes in plasma protein concentrations do show, therefore, after the 10-minute samples, the ebb and flow of protein from the stores to blood and of blood to stores. The small amplitude of this tidal movement, coupled with the fact that the values for the plasma proteins are high, suggests strongly that this animal, too, had well-filled protein stores.

Saturation of the protein stores can be accomplished by repeated plasma transfusions. No. 93, after 2 transfusions, showed a final plasma protein concentration of 8.57 g. per 100 c.cm. 56 minutes after the second transfusion, identical with the value at the start of the experiment. A third transfusion raised the level to 9.22 g. in 15 minutes, and maintained it there for a further 20 minutes. In No. 91, at 29 minutes after a third transfusion, the plasma protein concentration was only slightly higher than the level before the first transfusion. The fourth transfusion raised the level significantly and maintained it there for 1 hour. Saturation of stores was completed by the second transfusion in No. 106, and a subsequent transfusion raised the level still higher and maintained it for a further 40 minutes.

In view of Madden and Whipple's (1940) opinion that the liver is an important site for the storage of protein, it seemed possible to test on this organ the suggestions outlined above. Madden and Whipple envisaged a steady state, or ebb and flow, between the plasma proteins and those in body cells. That idea presupposes two reservoirs of protein, one in the blood stream and the other in reserve in tissue cells. A state of equilibrium between the two reservoirs tends to appear, and the speed with which it is accomplished will depend on the "gradient" between the "levels" of protein in the tissue cells and that in the blood stream. If the level of available plasma protein is low in the tissues, then high degrees of hypoproteinaemia may persist. On the other hand, a high level of protein in the blood stream will only remain high if the protein reserves are also high.

The isolated liver experiments show clearly that the liver can mobilize and throw into the perfusing fluid quantities of plasma protein (Tables V and VII, Nos. 150, 154, 164). The stimulus for this production is obviously the low plasma protein concentration of the perfusing fluid. There is, too, in these experiments a correlation between the degree of "hypoproteinaemia" and the rate of plasma protein production. One experiment (No. 165), in which the plasma protein concentration was low, showed no change in concentration over 50 minutes. It may be presumed that the reserves were also low in this liver, and this is supported by the fact that the addition of 1.10 g. of plasma protein produced a temporary rise and then a disappearance of all the added protein inside 1 hour. Equilibrium between stores and circulating protein seems to be suggested in one experiment (No. 153). Protein was lost from the perfusing fluid in two experiments (Nos. 172, 173). The loss was greater in the experiment with the higher plasma protein concentration.

When plasma was added to the perfusing fluid about 1 hour after the experiment had begun, the results obtained again seemed to depend partly on the state of the reserves and partly on the concentration of protein in the perfusing fluid. One liver (No. 164) showed a continuing output of protein after the addition of plasma, while another (No. 165), perfused

under precisely the same conditions with a perfusing fluid almost identical with that used in No. 164, showed a loss of the added plasma protein. Previous to the addition, this liver had added no protein to the perfusing fluid, and hence it can be presumed that the reserves in this liver were low. Another liver (No. 167), perfused with a rich plasma protein fluid, lost protein from the circulating fluid. The added plasma protein produced a rise in concentration, and presumably all of it remained in the perfusing fluid. The addition of Tyrode's solution merely diluted the perfusing fluid without producing any further addition of protein. The remaining experiment (No. 170) showed a small rise in concentration before plasma was added. The added protein remained in the perfusing fluid, although from the values obtained for protein concentration it would appear that there was at first a movement out of the perfusing fluid, followed by a movement in the reverse direction.

There can be no doubt, therefore, that the liver does behave as a "store" for plasma protein. It remained, however, to determine if it was in fact the only store. Hepatectomized animals were prepared and after variable periods given plasma transfusions. Two animals (Nos. 147, 158) showed no change in total plasma proteins before they were given a transfusion. One (No. 147), at 70 minutes after the transfusion, showed an appreciable loss of protein out of the circulation. The other (No. 158) lost a greater amount in 40 minutes. Another animal (No. 162) had lost some protein before transfusion and continued to lose an even greater quantity afterwards.

These results may indicate a movement of protein into other stores, but they are not as convincing as evidence of movement from such stores into the blood. Such evidence is provided by Nos. 161 and 166. Over a period of 30 minutes, while blood dilution was proceeding, No. 161 raised its total plasma protein by a small but significant amount. No. 166, over a period of 45 minutes, also while blood dilution was proceeding, raised its total plasma protein by 11% over the expected value. No. 161 continued to add protein to its circulation after a plasma transfusion at about the same rate as before transfusion.

The evidence from these experiments does suggest that plasma protein leaves the blood stream in hepatectomized animals in quantities greater than might reasonably be required for normal metabolism, and, conversely, these animals seem to possess some source of plasma protein other than the liver.

The speed with which protein may enter or leave the blood stream or perfusing fluid lends support to Whipple's view that protein can move as such from cells to plasma, and plasma to cells, as the need arises. It is clear, too, that the storage mechanism is not capable of dealing with large quantities of excess protein added to the blood. A rising plasma protein concentration after repeated plasma transfusions is an indication of saturation. The site of the protein stores cannot be pinned down exclusively to the liver. Whether all body cells can contribute protein to the blood stream with the facility with which they apparently can withdraw it has yet to be decided.

Summary

Repeated plasma transfusions show that the plasma reserve stores may become saturated with protein and that further transfusions will lead to a rising plasma protein concentration and plasma volume.

The liver can add to or abstract plasma protein from the blood. The direction of the movement depends on the state of the liver's reserves and the plasma protein content of the blood.

Protein stores from which plasma proteins may be derived, or in which plasma protein can be stored, exist apart from the liver.

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