turbance of pigment construction in pernicious anemia is indicated by this study, it is not possible to state whether the observed increased coproporphyrin I excretion is a simple increase of a normal process or is an abnormal or disproportional type of construction such as is present in porphyria.[11]

The levels of coproporphyrin I and urobilin excretions in pernicious anemia are similar to those reported for hemolytic jaundice.[12] Moreover, the changes in the rate of output after specific therapy in the two conditions are alike. These facts suggest that the increased coproporphyrin I excretion in pernicious anemia during relapse reflects a normally increased production proportional to an increased Type III porphyrin formation rather than a pathological production resulting in a disturbed ratio between the formation of Type I and Type III compounds.

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Intravenous Injection of Aminoacids in Regeneration of Serum Protein Following Severe Experimental Hemorrhage.

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Nine pairs of experiments were performed, dogs of the same size, sex and condition being used as far as possible for each experiment. Under local anesthesia 3.5% of the body weight was bled from the femoral artery and immediately the same volume of Ringer's solution replaced into the femoral vein. One half hour later the first specimen of blood was taken from the jugular vein. Each dog was then given intravenously over the course of 3 hours a volume of fluid equal to 3.5% of the body weight. To one, 10% glucose in Ringer's was given; to the other 5% glucose plus 5% aminoacid mixture in Ringer's. Six hours after the beginning of the injection a second sample of jugular blood was taken; a third specimen was obtained 24 hours later when the experiment was finished. Additional samples of blood were taken in all animals to determine the level of its aminoacid content. No symptoms were produced by the injections and the dogs remained in good condition throughout the period of observation. Though allowed water, all food was withheld, not only during the experiments but for a period of 48 to 72 hours previously. The slow rate of injection of the aminoacids raised the aminoacid nitrogen of the blood to not over 11 mg./100 cc.; one half hour after the end of the injection it returned to the normal level, about 6 mg. Only about 10% of the injected aminoacids appeared in the urine. The Folin colorimetric method was used for determining aminoacid nitrogen.

The blood samples were measured for cell volume and total protein. Hematocrits were done by centrifuging whole blood rendered incoagulable with prontamine fast pink Bl—one mg. per cc.; a second sample was centrifuged after adding 5 cc. to one cc. of 1.6% sodium oxalate. The values obtained checked usually within 1%, never greater than 3%. Serum protein was determined by the macro-Kjeldahl-titration method and was done in duplicate, checks within 2% being obtained. The value for non-protein nitrogen was always the same, but was first subtracted from the total nitrogen before calculating the protein value.

The mixture of aminoacids used was obtained through the courtesy of Mead Johnson and Company and with the continued coöperation of Drs. Cox and Kemmerer of their staff. It was prepared by hydrolyzing purified casein; tryptophane was added, 2% by weight, and the mixture dissolved in Ringer's solution and filtered before use. It formed a clear, light amber solution of a pH of 5.8, was not anaphylactogenic for guinea pigs and showed no precipitate when treated with the tungstic acid reagent.

CHART I.
Actual Serum Protein Changes* (gm. per 100 cc. serum or plasma).

	Glucose Injections			Aminoacid Injections		
Exp.	Start	6 hr.	24 hr.	Start	6 hr.	24 hr.
1	5.40	—.40	—.10	4.0	+.60	xx
2	5.30	—.10	+.10	4.50	+1.00	+.30
3	4.90	+.20	—.10	4.90	+.50	+.90
4	5.20	—.30	+.30	5.40	+.10	+.30
5	4.19	—.20	+.06	4.40	+.26	+.57
6	4.86	—.20	+.34	4.46	+.31	+.61
7	4.59	+.35	+.31	4.37	+.26	+1.40
8	4.68	+.30	+.35	4.72	+.22	+.79
9	4.24	—.44	+.13	5.11	+.18	+.31
Av.		—.09	+.15		+.38	+.64
%		—2	+3		+9	+14

*Changes are indicated by + or — and are obtained at both 6 and 24 hrs. by subtraction from the original ("start") value.

The findings are recorded in Chart I. It will be seen that whereas the animals treated with glucose alone showed no regeneration after 6 hours and little even after 24 hours, the dogs treated with aminoacids and glucose exhibited a definite rise in 6 hours and a marked one in 24 hours. Lest this increased value be due to a concentration of the blood it was necessary to consider any changes in the total volume of circulating plasma. Direct measurements of the plasma volume with the vital red technic did not give accurate results, owing to difficulty in colorimetric readings although no essential differences between the glucose and aminoacid animals were observed. To get an approximate figure for the total plasma proteins the hematocrit was used, assuming that the total cell volume did not change during the period of observation or at least that similar changes occurred in the glucose and the aminoacid animals. That no essential change occurred in the size or pigment content of the red cells was indicated by a close correlation between the erythrocyte count, hemoglobin value (Sahli) and hematocrit, all of which showed a similar drop indicating an increase in the relative plasma volume, *i. e.*, dilution of the whole blood. The results obtained by calculating the relative total plasma protein changes are indicated in Chart II; it

CHART II.
Relative Total Plasma Protein Changes* (% of initial value).

	Glucose Injections		Aminoacid Injections	
Exp.	6 hr.	24 hr.	6 hr.	24 hr.
1	—6	+8	+48	xx
2	+18	+25	+47	+42
3	+27	+31	+66	+78
4	+18	+21	+48	+39
5	+30	+40	+70	+87
6	+28	+45	+57	+54
7	+31	+33	+22	+51
8	+25	+30	+30	+61
9	+ 9	+32	+24	+40
Av.	+20	+29	+46	+57

*Changes are calculated at both 6 and 24 hours from the original ("start") value which is taken as 100%.

will be seen that here too the increase of plasma protein is far greater in the aminoacid dogs than in those receiving glucose alone. The method of calculation was as follows: for the initial value, the plasma volume (hematocrit reading) was multiplied by the serum protein; this was taken as the amount of protein per 100 cc. of blood at the start. Subsequent plasma volumes per 100 cc. of this original blood were obtained by multiplying the actual plasma volume observed in the hematocrit by a factor which corrected for the observed change in relative cell volume (hematocrit reading). Thus, if the relative cell volume changed from 35 to 30, the plasma volume (per 100 cc. of the original blood "at the start") changed from 65 to $70 \times 35/30$. The plasma volume thus obtained was multiplied by its serum protein concentration and the change expressed as a percentage of the initial value (Chart II).

Summary. Healthy starving dogs subjected to a single severe hemorrhage revealed a much more striking regeneration of serum protein in 6 and 24 hours when treated by intravenous injection of a complete mixture of aminoacids and glucose than of glucose alone. The findings suggest that the aminoacids introduced in this way are utilized in the regeneration of serum protein. Clinical observations in patients unable to take protein by mouth have given similar results; nitrogen balance observations on them as well as on animals are now being carried out.

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