EFFICACY OF PROTEIN HYDROLYSATE IN THE RESTORATION OF SERUM PROTEIN IN HYPERIMMUNIZED HORSES AFTER BLOOD DEPLETION

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(With 1 Figure in the Text)

Maintenance of blood volume and restoration of plasma proteins are essentials desired in the treatment of haemorrhage and surgical shock. Blood volume can be temporarily maintained by parenteral administration of glucose-saline, gum-saline, etc. But regeneration of plasma proteins, lost in haemorrhage, should be as quick as possible. Depleted protein reserve of the body can be supplemented by intake of adequate dietary protein. Parenteral feeding should be confined to conditions, where ability to utilize dietary proteins is seriously impaired and to urgent cases, where quick restoration of proteins is imperative. The animal body can fabricate the serum proteins only when the feed—oral or parenteral—contains essential amino acids. Beef and veal contain these amino acids in good proportions (Beach, Munks & Robinson, 1943). They are cheap and easily available. As such, a study of the efficacy of intravenous administration of an enzymic hydrolysate of these in restoring serum proteins after depletion of blood was undertaken.

EXPERIMENTS

Preparation of hydrolysate. Healthy animals were slaughtered under strict hygienic conditions. The meat, freed from fat and fasciae, was finely minced. The following materials were then placed in an enamelled drum:

Minced meat	15 kg.
Papain (Ceylon variety; emulsified in a small volume of pyrogen-free distilled water)	600 g.
Pyrogen-free distilled water up to ...	15 l.
Toluene	150 c.c.

The toluene was added after the temperature of the charge had been raised to 55° C. in a water-bath. The container was then incubated at 55° C. After 48 hr. the digest was steamed for 20 min. and filtered through paper. The filtrate was diluted with pyrogen-free water so as to contain approximately 4 g. nitrogen/l. With trichloracetic acid and with half-saturation with ammonium sulphate this hydrolysate gave no turbidity, showing complete absence of undigested protein and primary proteose. The reaction of the digest was adjusted to *p*H 7·4 and the whole autoclaved at 15 lb. for 25 min. It was then paper filtered. 5·0 % of glucose and 0·9 % of sodium chloride were added, and the solution distributed in sterile blood transfusion bottles and autoclaved at 10 lb. for 20 min. Samples were injected intravenously into rabbits (10 c.c./kg.). The animals showed no pyrexial reaction.

Administration of the hydrolysate. Twenty horses used for routine preparation of therapeutic antisera were used in the experiment. After one whole bleeding of 6 l., the volume lost was partly replaced in ten horses by intravenous transfusion of 1 l. of the above solution. The transfusion was repeated daily for the next 3 days. Samples of blood were collected on each day including the day of bleeding before the transfusion. Ten control animals were treated in exactly the same manner except that, in place of hydrolysate-glucose-saline, only 5 % glucose in normal saline was administered.

Analysis of serum samples. Samples of blood were collected and allowed to clot. The serum which separated was analysed for total nitrogen, non-protein nitrogen, albumin and globulin nitrogen (micro-Kjeldahl) according to the methods outlined by King, Haslewood & Delory (1937). The protein content was calculated by multiplying the nitrogen figure by 6·25.

The results obtained are recorded in Table 1.

Five horses were treated after bleeding with hydrolysate as above and the protein content of serum samples determined. The same animals were treated similarly after an interval of 1 month, but 5 % glucose in normal saline was injected in place of the hydrolysate. The results obtained have been statistically treated in Table 2. The protein contents of the samples of sera collected on 4 consecutive days have been shown in Fig. 1.

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Table 1

		Col. 1 Average of 10 figures			Col. 2 Statistical treatment of 1st and 4th day figures. Test of significance (T)		
		Protein % (nitrogen × 6·25)	Albumin % (nitrogen × 6·25)	Globulin % (nitrogen × 6·25)	Protein	Albumin	Globulin
With hydrolysate	1st day (m_1)	6·80	1·36	5·43	0·68 towards fall	0·96 towards rise	1·04 towards fall
	Standard error (E_1)	$\sqrt{0·0117}$	$\sqrt{0·007}$	$\sqrt{0·0196}$			
	4th day (m_2)	6·67	1·47	5·20			
	Standard error (E_2)	$\sqrt{0·0261}$	$\sqrt{0·00608}$	$\sqrt{0·0277}$			
	m_1-m_2	0·13	0·11	0·23			
Without hydrolysate	1st day (m_1)	7·15	1·41	5·75	3·90 towards fall	0·75 towards fall	3·11 towards fall
	Standard error (E_1)	$\sqrt{0·017}$	$\sqrt{0·00599}$	$\sqrt{0·0125}$			
	4th day (m_2)	6·5	1·32	5·19			
	Standard error (E_2)	$\sqrt{0·01}$	$\sqrt{0·0087}$	$\sqrt{0·0202}$			
	m_1-m_2	0·65	0·09	0·56			

Standard error, $E=\sqrt{\frac{\leqslant d^2}{n\,(n-1)}}$, where d = difference of individual figure from mean and n = number of figures in the series.

Test of significance, $T=\frac{m_1-m_2}{\sqrt{(E_1^2+E_2^2)}}$.

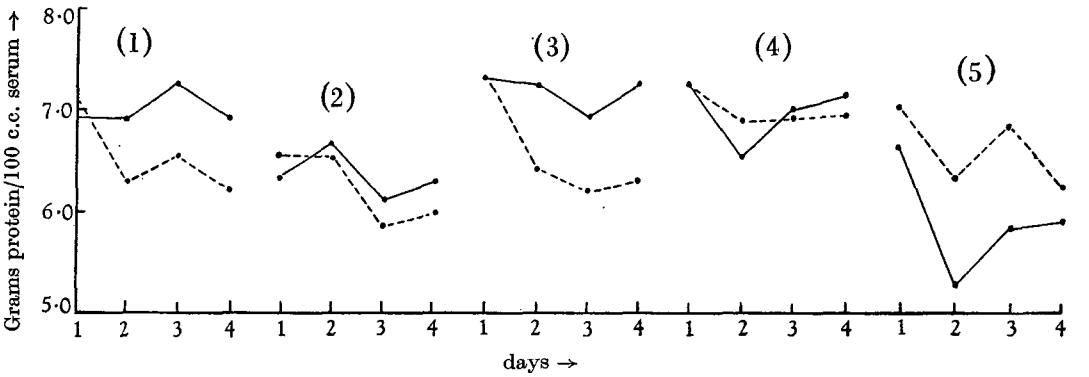

Fig. 1. Continuous lines indicate results with hydrolysate; dotted lines indicate results without hydrolysate.

Table 2

Figures indicate average results of the same five horses with and without hydrolysate.

		Protein % (nitrogen × 6·25)	Statistical treatment of 1st and 4th day figures. Test of significance (T)
With hydrolysate	1st day (m_1)	6·90	0·57 towards fall
	Standard error (E_1)	$\sqrt{0·0556}$	
	4th day (m_2)	6·70	
	Standard error (E_2)	$\sqrt{0·0687}$	
	m_1-m_2	0·2	
Without hydrolysate	1st day (m_1)	7·04	3·38 towards fall
	Standard error (E_1)	$\sqrt{0·01839}$	
	4th day (m_2)	6·34	
	Standard error (E_2)	$\sqrt{0·0246}$	
	m_1-m_2	0·7	

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DISCUSSION

It is evident from the results (Tables 1, 2 and Fig. 1) that within the limits of the experiment there is a significant fall (cf. Burns, 1937) of serum protein after bleeding. This fall is found to be prevented with hydrolysate. As regards serum albumin, there is some fall after bleeding, and there appears to be some rise with hydrolysate, but when the figures are statistically treated results are not found to be significant. There is a significant fall in globulin fraction after bleeding which is minimized with hydrolysate (Table 1).

The animals used in this experiment having been previously hyperimmunized, the globulin content was initially much higher than is found in normal horses (cf. Basu, 1946). Previous workers have observed (Whipple, 1942; Whipple & Madden, 1944; Madden & Whipple, 1940) that blood depletion more markedly affects the albumin fraction than the globulin fraction. Here, blood depletion as well as treatment with hydrolysate is found to affect the globulin fraction much more significantly. This is probably due to the fact that the system of the animals used in our experiments had previously received prolonged and strong stimulation for globulin production.

SUMMARY

1. Treatment with enzymic hydrolysate of protein after blood depletion has a significant action in restoring serum protein content.

2. Blood depletion of hyperimmunized horses alters the serum globulin fraction much more markedly than the serum albumin fraction.

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