

normal physiological manner. The results of 7 experiments are summarized in Table I and show significant regeneration of blood plasma proteins.

In one dog not included in the table, which received the digest intravenously over a period of 33 days, the blood plasma proteins were 4.93 at the beginning of the injection period (Hematocrit 40.2, red blood count, 6.7 M) and 4.82 (Hematocrit 30, red blood count 5.2 M) at the termination with a loss in weight of .7 kg. A second dog was obviously ill during the entire injection period of 13 days during which time the plasma proteins changed from 4.69 (Hematocrit 32, red blood count 5.8 M) to 4.66 (Hematocrit 20, red blood count 4.3 M), the loss in weight being from 8.8 kg to 7.73 kg. Death of the animal terminated the experiment.

Conclusion. Regeneration of plasma proteins was found to occur in dogs with depleted proteins stores and lowered plasma proteins on a nitrogen-free diet receiving intravenously an enzymatic hydrolysate of casein as the sole source of nitrogen. Parenterally administered casein digest constitutes an effective method of parenteral nutrition.

13541

The Form of Combination of Radioactive Iron and Copper in Plasma Following Ingestion.

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It has been generally accepted as a fact that iron in transport in the blood plasma following its ingestion occurs in combination with globulin. The use of radioactive isotopes enables us to distinguish between iron[†] and copper[‡] normally found and that recently added to the body economy. In earlier experiments we reported that, whereas

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† We are deeply indebted to Professor E. O. Lawrence and members of the Radiation Laboratory of the University of California for the radioactive iron used in these experiments, and in particular to Dr. M. D. Kamen, who was directly responsible for preparing the isotope.

‡ The radio copper was prepared in the cyclotron of the Department of Physics of the University of Rochester through the kindness of Dr. Gerhard Dessauer.

about 80% of the radio iron in plasma shortly after feeding was in the filtrate following precipitation of the proteins by trichloroacetic acid, nearly all of the iron in the red cell was in a form which was precipitated with this acid.¹ Below we tabulate further data relating to this subject.

It can be seen that in a longer series of observations the fraction of iron in the plasma which is not precipitated by trichloroacetic acid constitutes about 90% of the total iron (Table I) under the conditions of these experiments. Furthermore, the time after ingestion does not seem to be related to the amount of iron found in this fraction.

In Table II are listed the results obtained in an extension of these observations. By various procedures, the amount of iron in combination with protein was determined and found to be about 95% of the total. However, only about 15% of the iron was found to be actually bound to globulin as determined by half-saturation with am-

TABLE I.
Form of Combination of Radioactive Iron in Plasma Following Administration.
Trichloroacetic acid precipitation of proteins.

Dog	Wt, kg	Hematocrit, %	Dose of iron isotope		Form of iron salt	Route	Time after isotope in administr., hr	Fraction of
			mg	counts/min.*				isotope in filtrate, %
37-227	4.7	27	84	45,800	FeCl ₃	in diet	1	67
							2	77
							12	82
37-202	7.9	23	115	11,020	FeCl ₃	" "	1	100
							4	92
							6	98
							8	93
							10	94
37-202	11.9	19	58	25,400	Ferrie ammon. citrate	gavage	0.5	98
							0.75	91
							1	96
							3	88
37-202	8.8	22	55	66,500	Ferrie ammon. citrate		0.5	92
							1	91
							1.5	90
							2.5	85
38-112	10.0	26	122	12,400	Ferrous glueconate	intrav.	0.5	84
							2.0	92
Average of all determinations								90

*Activity expressed as counts per minute on a scale of four Geiger Müller counter.

¹ Hahn, P. F., Bale, W. F., Lawrence, E. O., and Whipple, G. H., *J. Exp. Med.*, 1939, **69**, 739.

monium sulphate. Since all but 10-15% of the iron appears in the filtrate following treatment with trichloroacetic acid, it is likely that that iron which is not bound as globulin is in rather loose combination and is quite easily split off. We have not determined[§] whether or not this latter fraction of the iron is merely adsorbed or in combination as a salt. Only about 5% of the plasma iron was dialyzable against distilled water using cellophane tubes. When the plasma was acidified, on the other hand, about 60% of the iron dialyzed. When

TABLE II.
Form of Combination of Radioactive Iron in Plasma Following Oral Administration.

Dog No.	40-149	39-196
Date	6/17/41	7/7/41
Dose, mg	0.93	4.1
Activity, counts per min.	1980	7450
Time after feeding, hr	1.7	2.2
Red cell hematocrit, %	25.5	19.5
Activity per 100 ml plasma	19.3	4.4
A Protein-bound iron, precipitated by $(\text{NH}_4)_2\text{SO}_4$, % of total	93	98
B Protein-bound iron, precipitated by 80% alcohol, % of total	74	94
C Protein-bound iron, precipitated by 4 volumes of acetone, % of total	—	95
D Globulin-bound iron, precipitated by $\frac{1}{2}$ saturation with $(\text{NH}_4)_2\text{SO}_4$, % of total	17	15
E Residue from dialysis against distilled water, % of total	—	8
F Trichloroacetic acid soluble fraction, % of total	79	—
G Dialyzable iron, % of total	3.5	7
H Dialyzable iron following acidification with sulphuric acid, % of total	59	—
I Dialyzable iron following addition of 1 mg of ordinary iron as FeSO_4	—	17

Procedures used in treatment of plasma for determination of form in which iron is combined. References by letter are to Table II.

A. Protein-bound iron: 10 ml of plasma + 10 ml of water + 20 g of ammonium sulphate. Filtered.

B. Protein-bound iron: 10 ml plasma + 50 ml 95% ethyl alcohol. Allowed to stand for 30 min. Filtered.

C. Protein-bound iron: 10 ml plasma + 40 ml of acetone. Filtered.

D. Globulin-bound iron: 10 ml plasma + 10 ml of water + 20 ml saturated sodium sulphate. Filtered.

E. Residue from dialysis: See G below.

F. Trichloroacetic acid soluble fraction: 10 ml plasma + 10 ml of 10% trichloroacetic acid. Stood 30 minutes. Filtered.

G. Dialyzable iron: 10 ml of plasma dialysed against 200 ml of distilled water in ice-box for 24 hours.

H. Dialyzable acidified: 10 ml plasma + 10 ml of water + 10 ml of 0.3 N H_2SO_4 dialyzed in cellophane for 24 hours.

I. Dialyzable iron after addition of neutral iron: 10 ml of plasma + 1 mg of iron as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ allowed to stand overnight. Dialyzed against 200 ml of distilled water in ice-box for 24 hours.

[§] Dr. Yoshikawa's stay being interrupted, it was considered advisable to put on record the observations as they stand.

ordinary iron of the order of magnitude of 100 times the amount present in plasma was allowed to stand in contact with plasma containing some of the tagged iron, exchange took place to some extent since subsequent dialysis yielded 17% of the isotope.

While these studies were being carried out some preliminary investigations² concerning the metabolism of radioactive copper were under way and some effort to determine the form in which copper is transported in the plasma was indicated. These experiments are summarized in Table III.

Dog 40-133 used in these experiments was a normal animal, whereas dogs 40-149 and 40-179 were anemic, having been made so by

TABLE III.
Form of Combination of Radioactive Copper in Plasma Following Ingestion.*

Dog	Time after feeding, hr	Activity conc. in plasma, total counts/100 ml	Activity conc. in fraction counts/100 ml	% of total		
A	Protein-bound copper: Precipitated by four vol. of acetone	40-149	3.6	2700	2634	97
B	Copper precipitated by 5 vol. of 95% alcohol	40-133	2.4	3790	3045	80
C	Globulin-bound copper: Precipitated by half-saturation with (NH ₄) ₂ SO ₄	40-149	1.7	3700	975	26
		40-133	2.4	3790	570	15
D	Trichloroacetic acid soluble copper	40-179	1.8	3460	2380	69
		40-133	4.2	3860	2500	65
E	Copper in diethyldithiocarbamate ether extr.	40-179	2.5	2800	1770	63
F	Dialyzable copper	40-149	1.7	3700	240	7

*Procedures used in treatment of plasma for determination of the form in which copper is combined. References by letter are to Table III.

A. Protein-bound copper: 5 ml of plasma + oxalate added to 20 ml of acetone and filtered.

B. Copper precipitated by alcohol: 2 ml of plasma + oxalate added to 10 ml 95% alcohol, allowed to stand 30 minutes and filtered.

C. Globulin-bound copper: 3 ml plasma + oxalate diluted with equal volume of saturated ammonium sulphate, allowed to stand 30 min. After centrifugation, the precipitate was washed with half-saturated ammonium sulphate before ashing.

D. Trichloroacetic acid soluble copper: 3 ml of plasma + oxalate were diluted with 3 ml of water and 3 ml of 20% CCl₃COOH added. After 30 min. the mixture was centrifuged and the precipitate washed with 5% CCl₃COOH.

E. Diethyldithiocarbamate extracted copper: 5 ml of plasma + oxalate to which was added Na₄P₂O₇ solution. After addition of 1.5 ml of sodium diethyldithiocarbamate reagent the solution was extracted with ethyl ether repeatedly and the combined extracts ashed for counting.

F. Dialyzable copper: 3 ml of plasma + oxalate was dialyzed against distilled water at room temperature for 24 hours in a cellophane membrane.

² Yoshikawa, H., Hahn, P. F., and Bale, W. F., *J. Exp. Med.*, in preparation.

repeated hemorrhage. The diets used consisted chiefly of white bread and salmon, providing a low iron intake, and have been described elsewhere.²

Nearly all of the copper is bound in some manner to protein. About two-thirds of the element is split off by trichloroacetic acid and about the same amount is removed in the diethyldithiocarbamate procedure, so the binding is apparently not a very firm one. As in the instance of plasma iron, only a small amount of the copper is found tied to the globulin fraction of proteins. The fact that such a small amount of the copper is in the dialyzable fraction suggests that very little of the metal is likely in an ionizable form. Again, as in the case of the plasma iron, the data do not show whether we are dealing with true salt formation or whether the protein tie-up is one of adsorption.

13542 P

Inhibition in the Slow Muscle of the Scallop, *Pecten circularis aequisulcatus* Carpenter.

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(Introduced by C. A. G. Wiersma.)

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The adductor muscle of *Pecten* consists of two parts, the larger of which is responsible for the rapid movements during swimming, and the smaller, which contracts slowly, is responsible for keeping the valves closed. The peculiarities of this muscle have been studied repeatedly.^{1, 2, 3} A satisfactory answer has not been given to the question of how the slow muscle can withstand rather large tensions for indefinite periods without appreciable fatigue and at the same time be able to relax very quickly when necessary.

Reflex Relaxation. In *Pecten* stimulation of the mouth in a normal animal leads to rapid relaxation of the slow muscle. In our experience the sensitive area for this reflex is much wider than hitherto

¹ Uexkuell, J. von, *Z. f. Biol.*, 1912, **58**, 305.

² Bayliss, L. E., Boyland, E., and Ritchie, A. D., *Proc. Roy. Soc.*, Series B, 1930, **106**, 363.

³ Bozler, E., *Z. vergl. Physiol.*, 1930, **12**, 590.