

sole source of protein was fibrin. Addition of riboflavin to the faulty blood diets did not prevent the cataracts, and cystine alone was not preventative when it made 7% of the dietary protein. There was, however, a definitely lower occurrence and intensity of the disease when both cystine (3.3%) and glutamic acid (15%) were added to the diet; and this preventative effect was still greater when glycine (3%) accompanied them. Incomplete prevention with the liberal supply of the three acids suggests inadequacy of some additional dietary component.

Summary. Two types of cortical cataract have been produced in salamander larvae by synthetic diets differing in their protein constituents. The first type, rarely detectable in the early stages and with a marked final shrinkage, was caused by a diet of purified casein and by diets of the separate muscle proteins. The second type, with an easily visible early zoning, large fluid content at maturity, and halted later changes, was caused by diets with high hemoglobin content. Prevention of the first type of cataract was attained by dietary supplementation with cystine, while prevention of the second type required all 3 components of glutathione and apparently some additional factor.

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Ferrous and Ferric Iron in Liver Extract.

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In recent years much attention has been paid to the relative efficiency of absorption and utilization of ferrous iron as compared with ferric iron in treatment of the iron deficiency anemias. It is not our purpose further to discuss this question, but rather to present the results of an investigation carried out on solutions of liver extract to which iron salts had been added.

In general, ferrous ions in solution are rapidly oxidized to the ferric state unless protected by some reducing agent. Borgen and Elvehjem¹ commented on the power of homogenized chicken liver extracts to reduce ferric iron. We had previously noted considerable reducing power in a simple aqueous liver extract. These facts sug-

¹ Borgen, D. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1937, **119**, 725.

TABLE I.
Ferrous and Ferric Iron in Liver Extract.

No. and description of sample		Total reducing power equivalent to mg Fe ⁺⁺ /cc	Total iron present, mg/cc*	Ferrous iron % of total iron
Z-34	Ferrous Sulphate added	20.5	6.35	97.3
F-34S	" " "	24.4	8.12	98.0
G-34S	" " "	23.5	8.32	96.3
G-35S	" " "	24.4	7.63	99.0
L-36S	" " "	17.2	8.04	98.0
Q-36S	" " "	—	9.67	98.6
I-35	Ferric Ammonium Citrate added	29.1	13.5 approx.	90.0
K-35	" " "	31.4	"	95.0
L-36	" " "	33.3	"	77.0
S-38	" " "	27.2	"	58.0
B-39	" " "	30.5	"	66.0
K-40	" " "	22.5	"	51.0
T-40	" " "	24.3	"	70.0
S-40	" " "	22.8	"	61.0
C-40	" " "	23.6	"	66.0

*Each cc of extract was derived from about 7.3 g of liver, and, before the addition of iron, had a specific gravity of 1.25 at 60°F.

gested the possibility that liver extract might prevent the oxidation of ferrous iron added to it and thus furnish a stable solution of ferrous iron for oral ingestion, at the same time combining any advantages inherent in liver therapy.

Procedure. In order to estimate the total reducing power of the liver extract used in this study, a titrimetric method was used, employing as oxidation-reduction indicator the Ortho-Phenanthroline Ferrous Complex and as oxidizing agent, a ceric sulphate solution. This method estimates all ferrous ions and in addition any other material whose reducing potential is less than 1.14 volts. As will be evident in Table I the total reducing power of the solution consists of that due to the ferrous ions present and an additional amount due to reducing substances in the liver extract itself. The results are expressed in terms of equivalent amounts of ferrous iron.

The determination of ferrous and ferric iron was carried out according to McFarlane,² with $\alpha\alpha$ Bipyridine and titanous chloride. The liver extract used throughout was a simple aqueous extract of pork liver. To 6 samples of this extract, processed on different dates from different purchases of liver, ferrous sulphate was added. Two to 3 weeks later analyses were made for ferrous and ferric iron. The results (Table I) show that the liver extract had effectively prevented oxidation.

The results of analyses for total reducing power suggested the

² McFarlane, William D., *J. Ind. Eng. Chem.*, 1936, **8**, 124.

possibility that ferric iron might be reduced to ferrous by the liver extract. A series of samples of commercial liver extract to which ferric ammonium citrate had been added* was analyzed for ferrous and ferric iron. The results (Table I) showed that 50 to 95% of the added iron had been reduced and was present as ferrous iron. The age of samples when analyzed varied from one month (C-40) to 4½ years (I-35).

Witts³ has referred to the "potentiating action of liver" in the therapeutic use of iron in secondary anemias. Barker and Miller,⁴ Cheney,⁵ and other investigators^{6, 7} have presented results which indicate that liver extract is a helpful therapeutic agent in the treatment of many cases of hypochromic anemia. The obvious suggestion arising from our experiments is that the reducing action of liver extract demonstrated here, by supplying or maintaining iron in the more efficiently utilizable ferrous state, may be, in part, the mechanism by which oral liver extract exerts whatever "potentiating" action it may have when used in combination with iron in the therapy of secondary anemias.

Summary. 1. An aqueous liver extract effectively prevented the oxidation of added ferrous iron. 2. Due to the reducing action of a commercial liver extract, from 50 to 95% of ferric iron added to it was later present in the ferrous state. 3. The efficacy of liver and iron combinations in the treatment of the iron deficiency anemias may be partly due to this action of the liver extract in supplying or maintaining iron in the more easily absorbable ferrous state.

* Solution Liver Extract with Iron Valentine.

³ Witts, L. J., *Lancet*, 1936, **1**, 1.

⁴ Barker, W. H., and Miller, D. K., *Am. J. Med. Sc.*, 1938, **105**, 287.

⁵ Cheney, G., *Folia Hemat.*, 1934, **52**, 31.

⁶ Sturgis, C. C., and Farrar, G. E., Jr., *J. Exp. Med.*, 1935, **62**, 457.

⁷ Keefer, C. S., and Yang, C. S., *Arch. Int. Med.*, 1931, **48**, 537.