*B. typhosus* (Craigie)[3]), absorptions were made with formolized strains.[†] Thus treated, only the strains that possessed the heat-labile factor absorbed from the Sonne phage and they were just as effective as the bacteria heated at 56°C.

These observations on the Shiga and Sonne phages indicate that while thermostable Sonne factors are involved in reactions with the former phage, the latter (homologous) phage reveals heat-labile bacterial components.

The Sonne phage reacted intensely also on a small number of strains of *B. dysenteriæ* Flexner, but suitable absorptive tests failed to reveal the presence of corresponding fractions of phage. The absorbing surface of one sensitive Flexner strain studied in its relation to the Sonne phages, in contrast to the absorbing Sonne strains, was found to be thermostable.

Although both Shiga and Sonne phages are capable of lysing a number of Sonne strains, they differ strikingly in thermal stability. After the Sonne phage was heated at 65°C. for one-half hour, its titer for the homologous Sonne culture and the susceptible Flexner strain was reduced from $10^{-10}$ to $10^{-5}$; after 4 hours at 65°C. the residual titer on both organisms was $10^{-1}$, and no lysis whatever could be demonstrated after 5 hours' heating. In contrast, even after 5 hours at 65°C. the titer of the rough fraction of the Shiga phage on either Sonne or rough Shiga strains was hardly affected.

## 9207

# Cobalt Content of Iron Compounds and Its Possible Relation to Treatment of Anemia.

E. J. UNDERWOOD.[*] (Introduced by E. B. Hart.)

*From the Department of Agricultural Chemistry of the University of Wisconsin, Madison.*

The physiological importance of cobalt has taken on greatly increased significance due to recent work in Australia and New Zealand demonstrating its value in the treatment of certain fatal diseases of sheep and cattle, which are characterized by progressive

---

[3] Craigie, J., and Brendon, K. F., *J. Path. and Bact.*, 1936, **43**, 233.

[†] The formolized bacteria were washed several times with distilled water to remove free formalin which inhibits phage action.

[*] Commonwealth Fund Service Fellow. On leave from the Department of Agriculture, Western Australia.

emaciation and anemia. Marston and Lines[1] reported success in curing "coast disease" of sheep in South Australia with doses of cobaltous nitrate supplying 1 mg. cobalt daily. Marked improvement in the live-weight, appetite and the anemic condition of affected animals followed the cobalt treatment. In the same year Underwood and Filmer,[2] working in Western Australia showed, by a fractionation method, that limonite ($Fe_2O_3.H_2O$) owed its potency in the cure of "enzootic marasmus" of cattle and sheep to its cobalt content. They had previously shown (Filmer and Underwood[3]) that its potency was not related to its iron content, since an iron-free fraction proved as effective as whole limonite. These workers showed further[2] that the disease in sheep could be cured with doses of cobalt chloride supplying as little as 0.1 mg. cobalt daily, and suggested that cobalt must be regarded as an essential element in animal nutrition. Marked increases in growth and improvement in the blood picture followed even this minute dosage with cobalt chloride. Filmer and Underwood[4] have fully confirmed these findings for sheep and have extended them to include affected cattle which respond to doses of cobalt chloride supplying only 0.3 to 1.0 mg. cobalt daily. They also report the interesting finding that the therapeutic action of sub-optimal doses of cobalt chloride was improved by the addition of traces of nickel.

Finally Askew and Dixon,[5] following on the above results, showed that the potency of the iron compounds used successfully in the treatment of "bush-sickness" of sheep in New Zealand was due to their cobalt content and reported success with minute doses of cobalt salts without added iron.

In view of these results it seemed likely that cobalt might be a very general contaminant of iron compounds, a factor of possible significance in the treatment of certain anemias where massive doses of such compounds are commonly used. Accordingly, a preliminary survey of a number of iron compounds, as found in this laboratory, was undertaken to ascertain their cobalt contents.

After separation of the iron as ferric chloride by continuous extraction of a strong hydrochloric acid solution of the iron compound with ethyl ether in a Kutcher-Steudel type of apparatus, cobalt was determined colorimetrically by the method of Kidson, Askew

---

[1] Marston, H. R., aand Lines, E. W., *Aust. J. Council Sci. and Indust. Res.*, 1935, **8**, 111.

[2] Underwood, E. J., and Filmer, J. F., *Aust. Vet. J.*, 1935, **11**, 84.

[3] Filmer, J. F., and Underwood, E. J., *Aust. Vet. J.*, 1934, **10**, 83.

[4] Filmer, J. F., and Underwood, E. J., *Aust. Vet. J.*, 1937. In press.

[5] Askew, H. O., and Dixon, J. K., *N. Z. J. Sci. and Tech.*, 1936, **18**, 73.

and Dixon.[6] Duplicate determinations and recovery tests with added cobalt showed this method to be particularly adapted to the type of materials being analyzed. No losses of cobalt were experienced during the extraction of the iron.

The results are given in Table I. They are expressed as parts per million of whole material and as parts per million of iron.

TABLE I.
Cobalt Content of Iron Compounds.

| Compound | | Cobalt ppm. whole material | Cobalt ppm. of Fe |
|---|---|---|---|
| Ferric chloride | USP (i) | 41 | 119 |
| '' '' | USP (ii) | 22 | 64 |
| Iron wire | C.P. | 40 | 40 |
| Ferrous chloride | A.R. | 13 | 47 |
| '' carbonate | C.P. | 22 | 45 |
| '' ammonium sulfate | C.P. | 2 | 14 |
| '' '' '' | A.R. | 14 | 98 |
| Iron and ammonium citrate | (i) | 3 | 18 |
| '' '' '' '' | (ii) | 16 | 96 |
| '' '' '' '' | (iii) | 12 | 72 |
| Ferric pyrophosphate | (i) | 0.4 | 2.3 |
| '' '' | (ii) | 0.5 | 2.9 |

Though the number of materials analyzed is quite small, it is evident from the selection given that cobalt must be considered a very general contaminant of all common iron compounds. The amount present varies tremendously according to the source of the material and may apparently bear no relation either to the chemical nature of the compound or to its "grade" of purity in the ordinary chemical sense.

Whether this contamination with cobalt has any physiological significance where iron compounds are used therapeutically (other than in diseases of sheep and cattle previously mentioned) is not at present known. This can only be ascertained by proper experimentation. It is suggested, however, that the presence of these traces of cobalt may have a more significant effect than has been recognized previously. This possibility is enhanced not only by the extremely small amounts of cobalt found to be potent in animal metabolism[2, 4] and its known stimulative effect on the hematopoietic functions as evidenced by the production of polycythemia in the rat,[7, 8] but also,

---

[6] Kidson, E. B., Askew, H. O., and Dixon, J. K., *Ibid.*, 1936, **18**, 601.

[7] Waltner, A., and Waltner, K., *Klin. Woch.*, 1929, **8**, 313.

[8] Orten, J. N., Underhill, F. A., Mugrage, E. R., and Lewis, R. C., *J. Biol. Chem.*, 1932, **96**, 11.

in the hypochromic anemias of man, by the massive doses of iron compounds used.[9, 10]

The reasons underlying the necessity for such massive doses of iron have been the subject of speculation for some time. The possibility that contaminants play a significant part has been suggested by Sheldon and Ramage,[11] and others, while Heath, Strauss and Castle,[12] Fullerton,[13] and Witts[14] suggest, as a result of their experiments, that the primary factor governing the dosage of these compounds is their capacity to undergo absorption in the intestinal tract. This is said to be governed normally by their solubility and ability to yield free ferrous ions. Though the paramount importance of iron deficiency in the hypochromic anemias can hardly be denied, it is difficult to explain some of the phenomena associated with their treatment entirely on this basis. The "threshold phenomenon" mentioned by Witts[14] may be cited as an instance. In this connection the recent work of Brock and Hunter[15] is of decided interest and would seem to eliminate poor absorption as the explanation of the large doses required. These workers conducted iron balance experiments which showed that, though the iron in ferric ammonium citrate and Blaud's pill was poorly utilized to form hemoglobin, it was readily absorbed and was retained in the body in considerable amounts. It is evident that low utilization in hemoglobin formation does not necessarily imply low absorption in the intestinal tract. In commenting on these results Brock[16] suggests that the massive dosage may be necessary either to supply essential elements which may contaminate the iron compounds or to facilitate the absorption of other materials in the intestinal tract. This latter suggestion seems rather unlikely in view of the fact that large amounts of iron are known actually to decrease the absorption of some elements, *e. g.*, phosphorus.

*Summary.* A selection of common laboratory compounds of iron has been analyzed and found to contain significant, though extremely variable, quantities of cobalt. The possible significance of this finding in the treatment of certain types of anemia is discussed.

---

[9] Sargent, W., *Lancet*, 1932, **222**, 230, 1322.

[10] Witts, L. J., *Proc. Roy. Soc. Med.*, 1931, **24**, 543; 1933, **26**, 607.

[11] Sheldon, J. H., and Ramage, H., *Quart. J. Med.*, 1932, **1**, 135.

[12] Heath, C. W., Strauss, M. B., and Castle, W. B., *J. Clin. Invest.*, 1932, **11**, 1293.

[13] Fullerton, H. W., *Edin. Med. J.*, 1934, **41**, 99.

[14] Witts, L. J., *Lancet*, 1936, **230**, 1.

[15] Brock, J. F., and Hunter, D., *Quart. J. Med.*, 1937, **6**, 5.

[16] Brock, J. F., *Brit. Med. J.*, 1937, **13**, 314.