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TRACE ELEMENTS IN RELATION TO HEALTH

Morning Session: Chairman, Dr. J. R. GREIG

Dr. J. R. Greig (Animal Diseases Research Association, Moredun Institute, Gilmerton, Midlothian): Since "pine" is perhaps the outstanding example of a mineral deficiency, it might be of interest to touch briefly upon some of the salient historical references to this disease.

The first mention of pining in the literature is probably that by James Hogg, the Ettrick Shepherd, in the *Quarterly Journal of Agriculture* (Hogg, 1831). Hogg even then realized that the disease was not infectious but was probably due to a nutritional deficiency. There appears to be no further reference to pine in the literature until 1922, when McGowan and Smith (1922) investigated the conditions of occurrence of pine on the northern slopes of the Cheviots. They suggested that the cause of the disease might be a vitamin deficiency although they recognized that some of the evidence did not support this view.

In 1928 Godden and Grimmett (1928) drew attention to the similarity between pining in Scotland and bush sickness in New Zealand, which was then believed to be due to a deficiency of iron. In the following year my attention was drawn to an acute form of pine which occurred on the island of Tiree. My colleagues and I found that the disease occurred only on those pastures of the island of which the subsoil was sand, and that it could be prevented and cured by administration of iron compounds, but it was a puzzling feature of this result that the iron content of the soil of the affected pastures seemed to be adequate. The same anomaly was noted by the Australasian workers on bush sickness, who later showed that the actual cause of the condition was a deficiency of cobalt, and it subsequently transpired that the curative and preventive action of iron compounds lay in their content of minute traces of cobalt as an impurity. It is now recognized that the Scottish form of pine is similar to, if not identical with, bush sickness in Australasia, "nakuruitis" in South Africa and "salt sick" in Florida.

In Scotland we have been engaged for some time in mapping out the areas on which cobalt pining occurs. It has recently been found in collaborative work between the Macaulay Institute at Aberdeen and the Animal Diseases Research Association at Moredun that the disease can be effectively prevented by dressing the pastures with artificial manures containing cobalt at the rate of 2 lb. cobalt sulphate per acre.

Although several considerable areas in Scotland are very deficient in cobalt and produce a severe form of pine, I believe that there are many other areas in which relatively minor degrees of cobalt deficiency occur so that sheep and cattle suffer from a sub-clinical form of pine, a condition of low health rather than of frank disease. An extension of cobalt

therapy would result in considerable improvement in the general health of the ruminant animals in such areas.

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Significance of Trace Elements in Relation to Diseases of Plants and Animals

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The purpose of this opening paper is to provide a bird's eye view of the knowledge of trace elements important in relation to plant and animal health, and to give a popular introduction to the more specialized papers which follow.

In biological usage the term "trace element" refers to any element which commonly occurs in minute amounts in living tissues, whether it is known to exercise any useful function or not. If such an element is found to be locally concentrated in some particular tissue, as in the case of iron in the red blood corpuscle or iodine in the thyroid gland, the tendency is to assume a purpose for it although it may be long before precise knowledge of function is gained. But when, as in the case of cobalt, the quantities are not only exceedingly small but distributed throughout the tissues with no known sites of predilection, the impulse is to treat their presence as fortuitous and unimportant until some instance of apparent absence is encountered in the form of an obscure deficiency disease or, as in the case of selenium, the effects of more substantial traces are manifested in the form of a toxicosis.

The plant, in absorbing essential major mineral nutrients from the soil, incidentally takes up traces of numerous other elements merely because they are brought into solution by the action of its roots. Some of these elements are inert, like nickel, which is always present in soil grown vegetation but without which the plant grows equally well in water cultures from which the element is carefully excluded. Others, for example copper, are beneficial if the traces are sufficiently small and yet harmful to some plants in water cultures containing as little as 1 part in 20 million. Some, like zinc, are perhaps inessential in the strictest sense of the term but definitely stimulate crop growth, and specifically increase resistance to plant disease. One at least, tungsten, appears to stimulate the early development of certain plants although excellent final growth is completed without it.

Since plant food is the basis of animal life, any trace elements present in plants naturally find their way into animal tissues but it may be long before they are recognized as essential, beneficial, harmful, or entirely inert. From the standpoint of animal health, assessment of significance is sometimes obscured by the fact that physiological function may be quite different in plant and animal economy, and that, even when a particular element is important for all forms of life, the quantities present may have a very different significance for different forms. For

example, the requirements of the plant for iron and copper are below those of the animal in the sense that a pasture may be growing luxuriantly and yet induce anaemia in the grazing animal. On the other hand, boron is a limiting factor in plant health but, if essential for animal life at all, the smallest quantities ever found in plants appear to be sufficient in the sense that no boron deficiency disease has been recorded in animals.

In selecting particular trace elements for special discussion, attention may be confined to those of proved economic importance. The elements copper, cobalt, manganese, zinc and boron may be taken as concerned with deficiency diseases, and fluorine, selenium and molybdenum as causing chronic intoxication. Iodine should of course be included, but is omitted on the ground that its more important functions are now widely known. Discussion of common trace elements such as aluminium, nickel, lead and arsenic is also omitted because they are not biologically essential and are not absorbed through plant roots in quantities injurious to animals. They may occur in foods or drinks in harmful amounts as contaminants but the amount of aluminium or nickel which is dangerous is too high to occasion alarm, while the dangers of contaminant arsenic or lead are too well known to call for comment. A few years ago foetal intoxication by lead, occurring naturally in pastures, was considered as a possible cause of congenital enzootic ataxia of lambs but copper deficiency in the pregnant ewes has since been incriminated. As for arsenic, the danger of cumulative poisoning is much exaggerated. Large, sub-toxic doses repeatedly administered to sheep are rapidly eliminated and the amounts naturally present in oysters and flat fish, although well above the limit sanctioned in foodstuffs by health authorities concerned with arsenic as a contaminant, are unconsciously consumed without detriment to human health.

Copper. The occurrence of copper in plant and animal tissues has been recognized for over a century, but it is only during the last decade that its importance has been conclusively established in agricultural and veterinary spheres. Since Professor Keilin will deal with physiological significance in the third paper this morning, present comments may be confined to disease aspects. In water cultures plants manage quite well without copper until blossoming, but seed formation is interfered with. The counterpart of this behaviour in natural conditions is the occurrence of deficiency diseases, of which the clearest cases have been recorded in Schleswig-Holstein and along the whole S.E. coast of Australia from Port MacDonnell to Meningie. In the former area the plant disease is called *Urbarmachungskrankheit* and is characterized by high incidence of "deaf ears" in cereals and by failure of root crops; associated with it is a disease of cattle termed *Lecksucht*, characterized by cachexia and pica. Fertilization of the soil with copper sulphate abolishes both diseases; weight of grain from oat and rye crops is much increased, lupins which fail to seed on untreated soil develop as normal plants, growing of sugar beet becomes profitable, young cattle develop normally and cachectic cows recover.

In the affected Australian coastal belt, application of as little as 14 lb. of copper sulphate per acre doubles the yield of oat grain, and benefit can still be detected 3 years after a single application. Associated animal diseases can be controlled by direct oral administration of copper or by

top dressing the pastures. The so called "falling disease" of cattle in West Australia, characterized by cachexia, anaemia, suppression of oestrus in cows, and stunted abnormal growth of calves, is regarded as a simple copper deficiency. Affected pastures show a copper content as low as 1 p.p.m. in the dry matter of the grass as compared with 5 to 15 p.p.m. for healthy pastures, on transference to which sick animals recover. The copper content of milk from affected cows may be as low as one-fifth of normal. Sheep also show symptoms, with a value for their milk as low as 0·001 mg. copper per cent. in highly deficient areas; in addition the cytochrome system of the tissues is reduced, and there is serious impairment of iron metabolism. Of more insidious character is the so called "enzootic ataxia" of lambs, a demyelinating disease affecting primarily the cerebrum of the foetus carried by outwardly healthy ewes and preventible by administration of small amounts of copper sulphate during pregnancy. The analogous disease, swayback, occurs in this country most notably in certain areas of Derbyshire, and is the subject of Professor Dalling's film this morning. The more acute forms of copper deficiency have not yet been encountered in Britain and there appears to be no parallel with the "falling disease" of Australia or with the *Lecksucht* of Schleswig-Holstein or its less pronounced analogue in Holland. In America there is a form of ataxia in foals bred on certain limestone soils which has, rightly or wrongly, been compared with swayback in sheep.

In indirect association with lack of copper come disorders such as "piglet anaemia," of world wide occurrence under intensive conditions of management and usually attributed to iron deficiency but perhaps also linked with a low content of copper in the milk of the sow. The phenomenal growth rate of the selectively bred modern domestic pig seems to have outrun the capacity of the udder to supply trace elements. The remedy for piglet anaemia is to paint the teats of the sow with iron and copper in treacle, and not to supply the elements to the sow herself. With regard to larger quantities of copper, it need only be mentioned that the gap between nutritional requirements and chronic intoxication is very wide; for sheep 5 mg. daily in the form of a soluble salt prevents disease but 100 mg. can be given with impunity for periods indefinitely long.

Cobalt. Since cobalt will be considered in three of the ensuing papers little need be said now. Although no definite relationship between plant growth and presence of cobalt has yet been established, and the amount of cobalt in the dry matter is only reckoned in parts per hundred million for leafy vegetation and parts per thousand million for cereals and meat, if we can trust our present analytical methods, yet the bio-economics of the element are impressive when considered in relation to the rearing of ruminants. "Coast disease" of sheep in south east Australia, "enzootic marasmus" of cattle and sheep in West Australia, "bush sickness" and Morton Mains "sheep ailment" in New Zealand, "Grand Traverse disease" or "lake shore disease" of cattle in Michigan, are all attributed to cobalt deficiency. Sometimes deficiency of cobalt is complicated by concomitant deficiency of copper, as in the case of Australian "wasting disease," as distinct from "falling disease" attributed to lack of the latter only.

Other diseases of ruminants, such as the "neck ail" of Massachusetts,

the "salt sick" of Florida, the "nakuruitis" of Kenya, and the "vinquish" of Scotland, formerly attributed to iron deficiency, are now being reconsidered in terms of trace elements in the wider sense. Fortunately where such deficiencies exist the remedy is simple; 0·1 mg. of soluble cobalt daily for sheep or 1 mg. for cattle is usually a sufficient rate of administration. In practice larger quantities at weekly or fortnightly intervals may serve the purpose. In some areas the deficiency is rectified by fertilizing the soil at the rate of about 2 lb. of cobalt sulphate per acre.

Manganese. The fact that manganese is essential is now well recognized although the quantity taken up by plants varies widely with soil character and with species, *e.g.*, 21 parts per hundred thousand of dry matter for *Dactylis glomerata* as against 5 parts for lucerne grown in the same conditions. Manganese deficiency is usually manifested by loss of green colour, affecting different plants in different ways. "Grey speck" of oats and wheat, "marsh spot" of peas, "speckled yellows" of sugar beet, and a variety of other diseases are now attributed to inadequate supply of manganese in available form.

From experimental work on rats, manganese is accepted as an element essential for animal life also, and it appears to affect growth rate, skeletal metabolism, ovulation, and development of the foetus. In general, however, there is enough in foods, especially in leafy plants, so that naturally occurring manganese deficiency disease is rare in animals. So called "slipped tendon" or "perosis" of intensively reared chicks has, however, been attributed to lack of manganese, has been produced experimentally with diets poor in manganese, and has been prevented by a manganese supplement, although other factors are probably also involved in its aetiology.

Certain forms of delayed ovulation in the larger farm animals, where the female comes into heat and accepts the male before descent of the ovum, have also been, rightly or wrongly, attributed to manganese deficiency occasioned by excessive liming of the soil, a procedure which reduces the availability of the soil manganese to the plant.

The toxic dose of manganese for animals is enormously high in comparison with nutritional requirements and, although chronic industrial manganosis has been reported in man, adverse influence on the grazing animal of a high natural manganese content in pastures has never been authenticated.

Zinc. Traces of zinc are essential for growth of certain moulds and, in the higher plants, the element stimulates photosynthetic activity and acts as a specific against certain diseases, such as "mottle leaf" of citrus plants, "little leaf" of fruit trees, and "bronzing" of tung trees. The yield of oat grain on the barren Robe sands in Australia is raised to 11 bushels per acre by fertilization with copper sulphate but to 18 bushels by accompanying application of zinc sulphate.

For animals an ordinary diet may well supply 0·1 mg. of zinc per kg. bodyweight so that no natural occurrence of zinc deficiency disease can be expected. Deficiency symptoms can, however, be induced in laboratory animals with synthetic diets; these include depressed growth, depigmentation of fur, hyperkeratinization of skin, and interference with purine metabolism.

Zinc tends to be distributed in sites of predilection, although the high value in the pancreas is not necessarily related to the observed retarding influence of simultaneously injected zinc salts on the hypoglycaemic effect of insulin.

A specific physiological function for zinc was found by Professor Keilin only two years ago, when he discovered that the enzyme, carbonic anhydrase, was a protein, free from iron, copper and manganese, but with the very high zinc content of 0·33 per cent.

Boron. Boron is essential for plant health and hence finds its way into animal tissues where, however, it is not known to serve any useful purpose. Quantities in plants range from about one part per million in cereals up to several parts per hundred thousand in potatoes and peas. The content is highest in the vegetative parts of plants, and forest leaves show relatively high values.

Since white rats complete the cycle of their being on experimental diets containing as little as 0·2 p.p.m. there is little likelihood of boron deficiency disease in animals. In plants, however, such disease is quite common. In wheat it is accompanied by abnormal tillering and defective setting of seed. In water cultures without boron, sugar cane exhibits depressed growth, distorted and chlorotic leaves, and definite stem lesions, but as little as 1 part of boron in 5 million of the nutrient solution establishes normal growth. "Brown rot" of turnips, "heart rot" or "dry rot" of sugar beets, and certain diseases of tobacco and other plants are attributable to boron deficiency. In Tasmania about 1 lb. of borax is used per apple tree every four years for prevention of "internal cork." In Australia "needle fusion" of pine trees also responds to treatment with boron.

Fluorine. Dr. Murray in the seventh paper today will doubtless discuss the inessential character of fluorine in human nutrition and its adverse effect in traces above the adventitious normal. In plants, a few parts per million are universally found, but they are not regarded as essential, nor do fluorine rich soils wreak havoc in the vegetable world. Quantities normally present in animal tissues vary from below 1 p.p.m. for blood up to about 250 p.p.m. for the dry matter of bone. Human urine normally contains about 1 p.p.m., bovine urine over five times as much, and the amount can be much larger before fluorosis is clinically apparent. If ingested in amounts much larger than the usual traces, however, fluorine seriously interferes with bone metabolism and can be deposited in enormous amounts, up to 10,000 p.p.m. or more.

Spontaneous fluorosis of animals is found in many parts of the world, the most notable diseases being the "darmous" of the rock phosphate areas of Algeria, and the "gaddur" of the volcanic soils of Iceland. Darmous is common to man and all the domestic animals. It is particularly common in sheep which die of inanition caused by the wearing down of the incisors and dystrophy of all the permanent teeth.

Fertilizers containing fluoride are not a source of danger since the amount passed through the plant is negligible, but the use of ground rock phosphate as a mineral supplement for cows has occasioned severe fluorine cachexia accompanied by reduced milk yield, lameness, exostoses on long bones and mandibles, hyperplasia of dental enamel, and other changes.

Occurrence of industrial fluorosis in animals has been reported in the vicinity of aluminium factories, where cryolite is used as flux, and Mr. Blakemore will describe this afternoon a severe outbreak in dairy cows in England, traced to deposition of fluorine compounds on grass in the direction of the prevailing wind from a group of brick factories.

Selenium. The inessential element selenium is biologically important only because it is vastly more toxic for animals than for plants, so that on seleniferous soils healthy plants can accumulate quantities of selenium high enough to produce disease in animals consuming them. Plants grown in water cultures in which the content of selenium in relation to that of sulphur is high show "snow-white chlorosis," but many plants can slowly extract from soils large amounts of the element without detriment to themselves, different species varying widely in this respect. Thus the botanically related *Astragalus missouriensis* and *Astragalus bisulcatus*, grown on the same soil, showed 3 and 1250 p.p.m. respectively. The latter plant is thus a selenium accumulator and also a selenium converter, in the sense that it readily absorbs the element from seleniferous shales and returns it in due course to the soil in more soluble form for absorption by other plants.

Selenosis has not been encountered in this country but in some parts of the world, notably in South Dakota west of the river Missouri, it has occasioned serious disorders in all stock, including poultry. The so-called "alkali disease" of this area, manifested by depressed growth, loss of hair most conspicuous in the mane and tail of horses, abnormal development and sloughing off of hoofs, is now known to be due to selenium poisoning. Amongst poultry a conspicuous feature is non-viability of eggs or hatching of monsters. The safe limit for the selenium content of grain is about 3 p.p.m., and the effect of a content of 10 p.p.m. in wheat on the hatchability of eggs is apparent from the seventh day onwards.

The "blind staggers" of range cattle in Wyoming is a more acute form of selenosis than the alkali disease of Dakota, liver lesions leading to death before loss of hair and hoofs occurs.

Although the element is widely distributed in nature it is quite readily excreted in bile and urine, so that no real danger exists until a level of chronic intoxication is reached, sufficiently high to produce irreversible damage to certain tissues. The urine of men employed in the extraction of selenium can contain up to a few p.p.m. without clinical symptoms appearing and the nutritional hazard from unintentional inclusion of selenized wheat in the milling grist is much reduced by the admixture with normal grain.

In known seleniferous areas, measures of control are now adopted to safeguard the health of stock and prevent abandonment of farms which have acquired a bad reputation. These include eradication of plants which are selenium converters, replanting with forages which do not accumulate selenium, improving soil drainage, avoiding cultivation of cereals for sale off the land and obviating overstocking. Fortunately most animals learn to avoid toxic plants, and some pastures are safe if understocked but dangerous if overgrazed.

Molybdenum. The element molybdenum which is widely distributed

in traces is not regarded as essential but, like selenium, it can be taken up by healthy plants in quantities injurious to animals. The clearest case of molybdenosis of farm stock is the so called "teart" of pastures on the molybdeniferous Lower Lias geological formation in Somerset. Since Mr. Ferguson is discussing this disease in the afternoon and will describe the classical observations from Jealott's Hill, it need only be mentioned that in some areas of Wyoming molybdenum is regarded as a contributory factor in the poisoning of livestock on range, and that a form of bovine haematuria in South Australia is at present suspected of being caused by long continued ingestion of molybdenum in quantities much smaller than those encountered in Somerset; the pronounced diarrhoea of teart is not observed and the haematuria develops very slowly.

The Distribution of Trace Elements in Soils and Grasses

Dr. R. L. Mitchell (The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen)

Trace constituents occur in soils in amounts from a fraction of one part per million upwards, and in different soils the amounts of any one may vary by as much as one thousandfold. Thus the variations in the amounts of the trace elements are greater than those of the major constituents. Before systematic relationship can be perceived in the amount of any trace element present in a given soil, it is necessary to go back to the material from which the soil is ultimately derived, that is, to the igneous rocks or even to the magmas from which these rocks crystallized. There are fundamental differences between magmas; thus it has been shown that, in general, the rocks of the area east of the Rocky Mountains are richer in barium and strontium than those of eastern Australia, pointing to a difference in the contents of these elements in the magmas. From a given magma, however, it is possible to say, at least for many of the important trace elements, whether they may be expected to be concentrated in a basic or an acidic rock. This is probably best illustrated by data for rocks of the Skaergaard Intrusion in Greenland, described by Wager and Deer (1939). There occurs here a series of rocks, whose character passes from ultrabasic, or silica poor, to acidic or silica rich, and all of them derived by fractional crystallization from the same original magma. A spectrographic examination of various members of the series has confirmed that the distribution of the trace constituents conforms with the existing knowledge of their geochemical behaviour.

The main factors governing the distribution of trace constituents in minerals have been elucidated chiefly by the specialized work of Goldschmidt and his colleagues, summarized by Goldschmidt, Barth, Lunde and Zachariasen (1926) and Goldschmidt (1937), who have shown that the probability of one element replacing another in a mineral depends on their relative ionic radii and valencies. If a trace element is to occur in a rock, there must be present in it a mineral one of whose constituents can be replaced atom for atom by the trace element in question. Nickel, for instance, may be expected to replace magnesium in olivines and

pyroxenes, as both are divalent and have ionic radii of 0·78A. Goldschmidt's description of the process is that a mineral which is separating from the magma sieves out those trace elements whose ionic radius and charge are similar to those of one of its major constituents. Thus the rocks formed at that stage are richer in these trace constituents than those formed previously or subsequently. For a few elements such as zirconium it appears necessary to postulate separation as oxides or similar compounds rather than inclusion in the crystal lattice of other minerals. It is impossible here to do more than indicate this effect by quoting in Table 1 results for one or two of the trace constituents in the Skaergaard series of rocks to show the variation on passing from basic to acidic rock types. A preliminary discussion of the results by Wager and Mitchell (1943) has now been published.

TABLE 1

DISTRIBUTION OF SOME TRACE CONSTITUENTS IN P.P.M. IN THE ROCKS OF THE SKAERGAARD INTRUSION, EAST GREENLAND

	Original magma	Ultrabasic							Acidic
		1	2	3	4	5	6	7	8
BaO ..	30	10	50	30	60	100	200	600	2000
Ga_2O_3 ..	33	13	27	20	27	20	27	27	20
NiO ..	120	750	250	30	<1	<1	<1	4	6
V_2O_3 ..	225	150	300	375	60	<7	<7	<7	15

Cobalt and chromium behave in much the same way as nickel, so that on this basis no deficiencies in nickel, cobalt or chromium would be expected in soils derived from ultrabasic rocks; in fact, effects due to nickel and chromium poisoning have been reported in the vegetation in some serpentine areas (Robinson, Edgington and Byers, 1935).

The problem is vastly complicated by the processes of weathering and metamorphism. In sedimentary rocks the changes occurring during the formation of the deposit or later are such that the behaviour of the trace constituents can as yet scarcely be foretold accurately, and quite unexpected accumulations may occur. Thus, in the course of the analysis of some 250 Scottish limestones, one in the neighbourhood of a quartz-dolerite sill has been found to contain up to 1 per cent. of boron although generally the content is below 10 p.p.m.

Hence, the distribution of trace constituents in soils depends to a great extent on parent material and its mode of formation. If the soil can be related directly to an igneous rock, the trace constituents may be foretold with reasonable accuracy, but where, as often happens in the north east of Scotland, the soil is formed from glacial drift derived from various sedimentary or metamorphic rocks, the problem is generally an individual one for each soil. The range of contents of some trace constituents in soils from this area is set out in Table 2. The values have been determined spectrographically, and data for a few important elements, such as zinc, are lacking because the sensitivity of the methods so far used is insufficient for the amounts occurring in the soils. Where the lower

limit is not specified it is likewise too low to be determined by the methods employed, which have been described already by the author (Mitchell, 1940).

TABLE 2

RANGE OF CONTENTS IN P.P.M. OF SOME TRACE CONSTITUENTS IN SOILS FROM NORTH EAST SCOTLAND

Ag	up to 2	Mo	up to 2
Ba	300 to 5000	Ni	5 to 2000
Co	up to 300	Pb	up to 1000
Cr	10 to 5000	Rb	100 to 2000
Cs	up to 500	Sn	up to 200
Cu	5 to 5000	Sr	50 to 5000
Ga	5 to 300	Th	up to 1000
Ge	up to 10	Ti	1000 upwards
La	up to 500	V	20 to 1000
Li	30 to 5000	Y	up to 500
Mn	200 upwards	Zr	50 to 1000

These data for the total amounts of trace element present in the soil are of limited significance for agriculture, as it is the amount that the plant can take up which is the important factor. The total content gives little more than a guide to the possibility of the occurrence of a deficiency or excess of a given element. The greater proportion of any trace constituent present in a soil is generally bound up in the crystal lattices of the minerals and is quite unavailable until released by weathering. The problem of obtaining an accurate chemical assessment of availability to the plant is difficult. So far, at the Macaulay Institute, extraction with dilute acetic acid has been employed. This gives values for the major soil constituents in reasonable correlation with field results, and is being used for trace elements also, with promising results for some of them, although for others, such as molybdenum, a neutral or alkaline medium seems preferable. The relative influence on plant growth of total and readily soluble mineral contents assessed in this way can be seen in Table 3 in which an area, part of which is infertile as a result of nickel poisoning of the plants, is considered. The differences in fertility are much more closely correlated with the amount of readily

TABLE 3

COMPARISON OF CONTENTS OF TOTAL AND READILY SOLUBLE NICKEL IN P.P.M. IN SOILS WITH A HIGH NICKEL CONTENT

	Readily soluble		Total	
	Fertile area	Infertile area	Fertile area	Infertile area
Layer 1 ..	11	88	412	725
2 ..	8	27	661	832
3 ..	11	45	871	537
4 ..	11	59	813	708

soluble, than of total, nickel in the soil. In each layer of the infertile area there is about 4 to 8 times as much readily soluble nickel as in the good area, but no differences of this magnitude are found in the total contents.

Another aspect of the problem, the effect of vegetation on the distribution of trace elements in soils, is also a factor of importance. If the crop is not removed those elements which are taken up by the plant will tend to accumulate in a readily soluble form in the surface organic litter at the expense of the lower layers of the soil. Zones of depletion or of accumulation of trace elements in the different horizons of the soil profile, resulting from normal processes of soil formation such as podzolization or lateritization, are also to be found but, as yet, it is not possible to generalize about the behaviour of the different trace elements.

On some soils, generally those rich in calcium carbonate, deficiencies in trace elements may occur in the vegetation although abundant amounts are present in the soil. This effect, generally described as lime induced chlorosis, is apparently connected with the *p*H value of the soil; it is common in parts of England but is of limited importance in Scottish soils. It cannot be considered as a true deficiency of a trace element in the soil because it can be remedied by changing the *p*H without adding the element in question, but its existence illustrates the complexity of the trace element problem.

In some instances at least, the content of trace constituents in herbage can be related to the amount of material in the soil available to the plant. Table 4 shows the relative uptakes of three trace constituents by different pastures and also the ratio of the uptake by the pasture, to the concentration in the soil, of the acetic acid soluble fraction of the trace constituent. The relative uptakes of cobalt and nickel are of interest, as the latter is apparently taken up preferentially by the plant. The arbitrary nature of the acetic acid extraction must, however, be kept in mind.

TABLE 4

AMOUNTS IN P.P.M. OF SOME TRACE CONSTITUENTS IN PASTURES AND IN THE SOILS ON WHICH THEY ARE GROWN

Acetic acid extract of soil			Pasture content			Ratio pasture/soil		
Co	Ni	Mn	Co	Ni	Mn	Co	Ni	Mn
0·19	0·92	67	0·07	2·00	66	0·37	2·2	1·0
0·63	1·10	40	0·15	2·19	101	0·24	2·0	2·5
0·59	1·31	38	0·18	3·68	148	0·30	2·8	3·9
0·22	0·48	39	0·06	1·02	59	0·27	2·1	1·5
0·68	1·34	33	0·22	3·62	52	0·32	2·7	1·6
0·26	0·58	58	0·06	0·85	56	0·23	1·5	1·0

While it is the trace element content of a mixed pasture which is, in general, considered, it must be remembered that the different species of pasture plants have different uptakes. Table 5 gives the contents of cobalt, nickel and molybdenum in different plants grown in the same soil in pot culture. With differences of the order shown in the Table between the different species which constitute a mixed pasture, variations in the trace element content found may be due to any of three causes: varying content in the soil, varying composition of the pasture, or improper sampling when the different species are taken in the wrong proportion

TABLE 5

UPTAKE OF COBALT, NICKEL AND MOLYBDENUM AS P.P.M. OVEN DRY WEIGHT OF DIFFERENT PASTURE SPECIES IN POT CULTURE FROM A SOIL WITH A LOW COBALT CONTENT

	Co	Ni	Mo
Rye grass	0·18	2·64	0·86
Cocksfoot	0·06	1·58	0·49
Timothy	0·11	1·58	0·43
Red clover	0·18	2·32	0·34
Mixed pasture	0·14	2·22	0·34
Oat straw	0·03	0·64	0·45
Oat grain	0·03	1·86	0·39

Time of sampling and degree of maturity in the plant may also be important factors. Preferential grazing may leave those species which the animal does not eat and, thus, analysis of the pasture may give a wrong impression of the composition of the diet. The data given in Table 5 for the nickel content of oats illustrate the point that different parts of a plant contain different amounts of the trace element. The variation in uptake between different species might, in favourable circumstances, be used to overcome deficiencies or excesses of any element. Generally, however, a deficiency will be overcome by the application of the necessary element to the soil. In order to obtain even distribution of the very small amounts necessary, the trace element is best applied to a pasture in a top dressing of superphosphate or sulphate of ammonia. Cobalt deficiency in pasture, leading to cobalt pining in sheep, is an example of a deficiency which has apparently no effect on the health of the crop and cannot, therefore, like some other plant deficiencies, be diagnosed by visible leaf symptoms,

It has been found that, for the cobalt deficient pastures in Rossshire, a dressing per acre of 2 lb. cobalt chloride or sulphate, equivalent to 0·25 p.p.m. cobalt, will prevent and cure pining in sheep for two seasons at least, the cobalt content of the herbage being raised to a value considerably above that at which pining occurs (Stewart, Mitchell and Stewart, 1941, 1942). Table 6 shows the effect on the herbage, over a subsequent period of more than two years, of different dressings of cobalt. The higher amounts of cobalt would not be used in practice. If a value of 0·10 p.p.m. cobalt in the herbage is taken as being the upper limit for the occurence of cobalt pining, and the true value is probably rather less than this, it is seen that 2 lb. of cobalt chloride per acre raised the content considerably above this figure for two seasons, but in the third the effect was small except when larger amounts of cobalt had been added.

From the results which are so far available it appears that diagnosis of cobalt deficiency can be made as successfully by soil analysis as by pasture analysis.

Finally, in Table 7 will be found the range of contents of some trace constituents in pastures from the north east and north of Scotland. The values for iron have been included as it is desired to draw attention to the dangers of contamination of herbage samples by soil. A properly

TABLE 6

EFFECT OF APPLICATION OF COBALT TO THE SOIL ON THE COBALT CONTENT OF MIXED PASTURE HERBAGE EXPRESSED AS P.P.M. SOIL OR P.P.M. OVEN DRY HERBAGE

$CoCl_2 \cdot 6H_2O$ lb. per acre	Cobalt applied p.p.m.	Cobalt in soil: total p.p.m. (*a*)	Cobalt in soil: readily soluble p.p.m.			Cobalt in pasture p.p.m.		
			(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)
0	0	2·9	0·20	0·16	0·21	0·12	0·09	0·06
1	0·125	2·9	0·22	0·23	0·23	0·38	0·11	0·06
2	0·25	3·2	0·25	0·27	0·29	0·67	0·25	0·07
10	1·25	4·1	0·48	0·76	0·96	1·96	0·60	0·26
40	5·0	5·8	1·75	1·38	4·08	3·94	1·90	1·29
80	10·0	9·6	1·96	4·86	7·96	8·17	3·20	2·96

Time of sampling after application (*a*) 10 weeks
(*b*) 15 months
(*c*) 27 ,,

sampled pasture appears never to contain more than 150 p.p.m. of iron and, generally, the values which we have found are from 50 to 80 p.p.m., yet, in the literature, values of up to 1500 p.p.m. are recorded. Such values must indicate severe contamination with soil, and it is quite useless to determine trace constituents in such samples, as errors of up to 1000 per cent. may result. Even light contamination with 0·05 per cent. of soil on the fresh weight will give an increase of 100 p.p.m. in the iron content of the oven dry matter and might raise the apparent cobalt content of the herbage from 0·04 p.p.m. to 0·06 p.p.m., on the assumption that the soil is cobalt deficient, containing 5 p.p.m. of total cobalt and 4 per cent. of iron. Contamination of herbage samples to this extent appears to result from the normal operations of haymaking and may occur as a result of grazing. The results given in Table 7 are of a preliminary nature and

TABLE 7

CONTENTS IN P.P.M. OF SOME TRACE ELEMENTS IN PASTURES FROM NORTH EAST SCOTLAND

Ba	10 to 30	Mo	0·3 to 6·0
Co	0·03 to 0·6	Ni	0·5 to 4·0
Cr	0·1 to 0·5	Rb	5 to 15
Cu	2 to 14	Sr	4 to 20
Fe	50 to 150	Ti	2 to 15
Li	0·05 to 0·15	V	0·3 to 0·7
Mn	20 to 300	Zn	5 to 40

data for several elements which are possibly of significance, such as tin and lead, are not yet available.

We have generally found that the spectrographic method is the most suitable for measuring trace constituents in soils and pastures, as it is possible by suitable technique to determine the content of several elements simultaneously, with an accuracy at least as great as that obtainable by other methods.

It is not possible to discuss the distribution of individual elements in

the soils and pastures of Great Britain, because sufficient data are not available. Such figures as are available for plants concern areas with known plant or animal diseases directly attributable to the trace element content. Plant diseases reported include boron deficiencies, often lime-induced, of turnips and sugar beet in various parts of the country, manganese deficiencies on shell sand deposits leading to oat sickness in north Scotland, and similar deficiencies on lime rich areas of southern England and in the fens on peat. In the chalk country other deficiencies have been reported, generally in fruit trees. Copper deficiencies of herbage may be associated with swayback in sheep; a similar complaint occurs in Australia with herbage containing less than 4 p.p.m. of copper. Excess of molybdenum in Somersetshire pastures containing 10 or more p.p.m. is responsible for teart in cattle. Deficiency of cobalt in pastures containing less than from 0·04 to 0·08 p.p.m. leads to a form of pining in sheep reported in various parts of the country, and definitely confirmed by the Animal Diseases Research Association and ourselves in the northern counties of Scotland.

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Trace Elements in Relation to Physiological Function and Enzyme Systems

Professor D. Keilin and Dr. T. Mann (Molteno Institute, University of Cambridge)

The significance of trace elements for plants and animals can be investigated in several ways:

(1) The presence of these elements in different organs or tissues can be recognized and their concentration estimated.

(2) Their source and availability in nutrient material can be determined.

(3) The pathological conditions due to their deficiency or excess in the organism can be investigated and, finally,

(4) The function and the physiological properties of these elements within the organism can be determined.

It is only in this last line of work that the writers have some personal experience.

Of all the metals occurring in living organisms, a definite physiological function has so far been found for three only, iron, copper and zinc; to these may perhaps be added also magnesium, which is the metal of the prosthetic group of the chlorophyll molecule. Each of these elements

may exhibit several distinct functions even within the same organism. It is well known, for example, that iron is an essential element of blood haemoglobin, of muscle haemoglobin, of each of the 4 components of the cytochrome system, of catalase and of peroxidase. It is still more interesting that the same cell, such as that of heart muscle, may contain at least 6 of these physiologically important iron compounds.

These 3 elements have definite physiological properties only when they are bound stoichiometrically with well defined and highly specific protein molecules. This combination may be either a direct one or through an organic molecule such as porphyrin.

Although the protein itself is inactive, it nevertheless determines not only the magnitude but also the nature of the physiological properties of each of these metals.

With these few important points in mind the physiological significance of iron, copper and zinc can be briefly examined.

Physiological Function of Iron

Iron is the best known metal in living organisms. It is interesting to note that, with the one exception of a very rare respiratory pigment, haemerythrin, iron always appears, wherever its physiological role is definitely established, as an *iron-porphyrin-protein* compound. In this complex compound the iron-porphyrin nucleus, known as haematin, forms the active prosthetic group of the molecule.

Two categories of biologically important substances, the oxygen carriers and the oxidizing catalysts or enzymes, belong to the iron-porphyrin-protein compounds.

Oxygen carriers are substances capable of combining loosely with molecular oxygen, taking it up in places where it is available and giving it up where its pressure is low and where it is being used up. To this family of compounds belong the different haemoglobins of vertebrates and invertebrates, muscle haemoglobin, and chlorocruorin, the green haemoglobin of some marine worms.

To the *oxidizing catalysts* belong the 4 components of the cytochrome system which play a fundamental part in intracellular respiration. They are responsible for the transfer of hydrogen from the activated molecules of foodstuff to the molecular oxygen. They catalyse, therefore, the reaction of the combustion of hydrogen to water which liberates the large amount of energy necessary for the life of the cell.

To the family of iron-porphyrin-protein compounds belong also two other enzymes, peroxidase which catalyses the oxidation of substances by means of H_2O_2, and catalase which catalyses the decomposition of H_2O_2 into molecular oxygen and water.

This brief enumeration of the iron compounds illustrates the remarkable fact already mentioned, that the same iron-porphyrin nucleus when combined with different proteins forms a series of substances, like haemoglobins, some components of cytochrome, peroxidase and catalase, which have different properties and functions although they may be found within the same cell.

One of the important properties of all iron-porphyrin-protein compounds is that they are pigments and often show very strong and selective absorption spectra. This property greatly facilitates the study of the

mechanism of their action, as, for instance, for cytochrome, the activity of which can be observed in living, intact cells.

Physiological Function of Copper

Copper like iron forms with proteins two important groups of compounds, oxygen carriers and oxidizing catalysts.

To the *oxygen carriers* belong different haemocyanins, the blue copper-protein compounds of some Arthropods and Molluscs which, like haemoglobins, combine loosely with molecular oxygen, giving it up in a non-activated state in places of low oxygen tension.

To the group of *oxidizing catalysts* belong the copper-protein enzymes, such as phenol oxidases or ascorbic acid oxidase, present mainly in plants, Arthropods and some Molluscs.

The mono- or polyphenol oxidases are enzymes catalysing the oxidation by means of oxygen of substances like *p*-cresol, tyrosine, catechol hydroquinone and *p*-phenylenediamine. These substances, on oxidation, give highly coloured compounds. It is the sudden contact between these enzymes and corresponding phenolic substances which causes the darkening of the tissues of fruits, potatoes or mushrooms when they are bruised or crushed; the same reaction causes the darkening of the latex of the lacquer tree on exposure to air, and of the cuticle of insects after emergence from the pupa.

Another copper-protein compound is haemocuprein, which we have recently obtained in the pure crystalline state from the red blood corpuscles of mammals. No definite function can as yet be ascribed to this compound, but it is known that copper is essential for the formation of haemoglobin and for the development of cytochrome *a* in yeast cells.

Physiological Function of Zinc

Zinc, like iron and copper, is widely distributed in nature, but we have so far found only one enzyme containing zinc as its active group. This zinc-protein enzyme is carbonic anhydrase, which catalyses both phases of the reversible reaction $H_2CO_3 \rightleftharpoons CO_2 + H_2O$; it is present in large concentration in erythrocytes, in certain cells of the gastric mucosa and in the pancreas. It plays an important part in the transport of carbon dioxide by the blood, and controls generally the acid-base equilibrium of the body.

Although all the zinc in the red blood corpuscles belongs to carbonic anhydrase, yet the distribution of this metal in cells and tissues of different organisms is much wider than that of carbonic anhydrase. This clearly shows that, like iron and copper, zinc forms with different proteins compounds which have very different physiological functions.

One of the most important results obtained in these studies is that the physiological significance of the metals we have examined is apparent only when they are bound stoichiometrically to highly specific proteins (see Table 1). It is this specific protein which determines the nature and magnitude of the physiological activity of the metals. In fact, it depends on the protein portion of the molecule whether the same metal will act as a carrier of molecular oxygen, or as a catalyst promoting the oxidation of the substrate by molecular oxygen or by peroxide, or will simply split the last into molecular oxygen and water.

TABLE I

IRON, COPPER AND ZINC IN ENZYME SYSTEMS

Fe	Iron-protein	Oxygen carrier	Haemerythrin		
	Iron-porphyrin-protein	Oxygen carriers	Haemoglobins		
			Myoglobin		
			Chlorocruorin		
		Oxidizing catalysts	Cytochrome		
			Peroxidase		
			Catalase		
Cu	Copper-protein	Oxygen carrier	Haemocyanin		
		Oxidizing catalysts	Phenolic oxidases	Polyphenol oxidase	
				Tyrosinase	
				Laccase	
			Ascorbic oxidase		
		Unknown function	Haemocuprein		
	Copper-porphyrin	Excretory substance	Turacin		
Zn	Zinc-protein	Catalyses reaction $H_2CO_3 \rightleftharpoons CO_2 + H_2O$	Carbonic anhydrase		

Further, the magnitude of the catalytic activity of the metal when in combination with its specific protein is millions of times higher than that of the free metal itself. In fact, for kinetic experiments with pure enzyme preparations, such as catalase, phenol oxidase and carbonic anhydrase, we use only 0·1 to 0·5μg. of dry weight of enzyme protein. This corresponds approximately per ml. fluid to 0·0003μg. iron of catalase, 0·005μg. copper of phenol oxidase and 0·005 μg. zinc of carbonic anhydrase. The activity and properties of purified metallo-protein enzymes can be determined in dilutions at which neither the protein nor the metal can be detected by any known chemical or physical methods.

As the protein portion of the molecule plays such an important part in the activity of the metallo-protein complex we cannot dismiss the possibility that not the amount of the metal only but of the specific protein also may become the limiting factor in the formation of these physiologically important and highly active metallo-protein compounds.

Discussion

Sir J. Barcroft (Physiological Laboratory, Cambridge), opener: The papers just presented have given an account of the occurrence of trace elements in the soil, in plants, and in animals. The question which I ask myself is "What is a trace element?" Can we arrive at any definition which will clarify our ideas about a rather vague description? At the present time it does not seem possible to give a watertight definition of a trace element, but it is possible to suggest an experimental definition, one which may in the end turn out to be not quite correct, but which can be used for the time being, and checked against new information as it accumulates to see to what extent it meets the facts.

The first suggestion is that a trace element should usually be a metallic element, fluorine and iodine being exceptions.

The second suggestion is that it should be a normal constituent of the animal or plant. This requires a little more thought. A consideration about cobalt will serve as illustration. On a recent visit to some of the pining area in Rossshire I was assured that if half a field on the cobalt deficient land was treated with cobalt, and the other half left, the sheep would single out the treated land and feed upon it, while leaving the other. In discussing this point, my friend, Professor Keilin, pointed out that this did not necessarily mean that the sheep could sense cobalt directly, but merely that they might taste something in the grass for which the cobalt was responsible, just as he could tell from the colour of a cut potato whether the potato contained active copper, not because he saw the copper, but because the presence of minute quantities of copper was responsible for the blackening of the potato. Reasoning on similar lines one might question whether cobalt was actually necessary to the sheep at all, or whether pining might not be due to the presence or absence of something in the grass for which the cobalt was responsible. I do not think Mr. Lyle Stewart will accept that idea, nor would my friends in Scotland, because feeding the sheep with cobalt will cure pining. The point, then, is that the trace element must be something which actually operates in the organism.

The third point is the quantitative one. The trace element must be present in the right amount, and mischief arises in some cases if there is too much, as with molybdenum, or in other cases because there is too little, as with cobalt. Trace element diseases are not necessarily deficiency diseases.

Whatever the amount it must be a trace and that, I will define tentatively, as not more than one part in twenty thousand of the organism, approximately the proportion in which zinc and iron are present in the tissues of man. It might be said that iron in the human body amounts to about one part in ten thousand, but is all the iron there present as a trace element? Dr. Mann and Professor Keilin have just described the functions of iron in the body, and I know that their view would be that the iron in the blood, which is half of the whole amount in the body, is not there as a trace element because its function in haemoglobin is that of a carrier, not of a catalyst.

This last consideration brings me to my fifth and last point, that the action of a trace element should be essentially that of an enzyme.

My experimental definition therefore covers five points. The trace element is an element, probably a metal. It must be present in the tissues of the organism. It must be a trace, not more than one part in twenty thousand of the tissue.

Impairment of function must arise from some quantitative deviation from the body's normal content of it.

The trace element must form the basis of some sort of enzymic action.

Mr. A. L. Bacharach (Glaxo Laboratories, Ltd., Greenford, Middlesex): Is there any linked enzyme system in which the absence of one co-enzymic metal might, by breaking the chain, precipitate symptoms strictly due to the absence of a neighbouring metal, as probably occurs with the co-enzymic water soluble vitamins?

Dr. T. Mann replied: The deficiency of one metal may result in that of another one. Thus, for instance, copper is needed for the formation of haemoglobin and an anaemia may result, not from iron, but from copper, deficiency. Copper is also essential for the formation of cytochrome *a*, an intracellular iron catalyst.

Dr E. C. Owen (Hannah Dairy Research Institute, Kirkhill, Ayr): Does Dr. Green know of the work of Bach and Chodat on molybdenum as essential for fixation of atmospheric nitrogen and of the function of fluorine in the normal formation of teeth? Is selenium always a toxic element in view of its concentration in indicator plants such as *Astragalus* and in view of its stimulating effect on Tenmarq wheat?

Dr. H. H. Green replied: I am aware of the claim that molybdenum stimulates bacterial nitrogen fixation but not aware that it is regarded as essential.

The function of fluorine in formation of dental enamel will be dealt with by Dr. Murray.

With regard to selenium, plants grow normally in water culture without it and it has, therefore, been regarded as not essential. In traces it may act as a stimulant for some plants, although I am not familiar with the work referred to; the main economic significance of selenium arises from the toxic effects produced by excess.

Dr. C. A. Mawson (Royal Berkshire Hospital, Reading): Is there any evidence of protein deficiency anaemia?

Dr. T. Mann replied: Hahn, Whipple and their coworkers in the United States have produced in experimental animals an anaemia due to deficiency of protein in the diet (Hahn and Whipple, 1939).

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Enzootic Ataxia or Swayback of Lambs in England in Relation to Copper Feeding of Ewes during Pregnancy

Professor T. Dalling (Veterinary Laboratory, Ministry of Agriculture, Weybridge, Surrey)

A film was shown illustrating the inco-ordination of the limbs of affected lambs attempting to keep pace with their mothers on pasture, and also the histological lesions found in the disease, particularly the demyelination of the cerebral hemispheres. The film was prefaced by the following introduction, read, in Professor Dalling's absence, by Dr. H. H. Green:

Swayback or enzootic ataxia is a nervous disorder of newly born and young lambs, occurring in different parts of the world, including Britain, Australia, New Zealand, South America, Sweden, South Africa and India. The incidence of the disease varies but may be as high as 90 per cent. The symptoms are usually evident at birth and are essentially those of spastic paralysis of the limbs with resultant inco-ordination. Occasionally blindness is present. The characteristic pathological change is a diffuse

symmetrical demyelination of the cerebrum, varying in extent in different cases from small foci in the *centrum ovale* to gross demyelination of the whole hemispheres. Liquefaction and cavitation are common end stages of the lesions. Secondary degeneration of the motor tracts in the cord is always present.

All the evidence points to the disease being of prenatal origin; the ewes which produce affected lambs show no obvious disturbance of health. While the causal agent has not been established, it seems to have a special affinity for the cerebral myelin and probably for the mechanism or cells responsible for the laying down of myelin.

Copper would appear to play a part in the aetiology although the manner in which it acts is still obscure. It has been shown that the copper values for the blood of affected lambs and their mothers are lower than those found in control animals. The feeding of copper in the form of copper sulphate to pregnant ewes prevents the occurrence of the disease in their offspring. In order to understand the aetiology more fully, fundamental studies of copper metabolism in the sheep and a more complete knowledge of the normal process of myelination in lambs are necessary.

Diseases of Stock in Australia Caused by Deficiency of Cobalt and of both Cobalt and Copper

Sir C. J. Martin (Roebuck House, Old Chesterton, Cambridge)

Introduction

Australia is not the only part of the world where stock waste and die for lack of a trifle of cobalt, but it happens that it was there found out that cobalt is essential to one or other of the metabolic processes in the nutrition of cattle and sheep. The amount required is small and of the order of 0·1 mg. daily for sheep and 0·5 mg. for cattle. The discovery was made simultaneously by two groups of investigators, Filmer and Underwood in Western Australia (Filmer, 1933; Filmer and Underwood, 1934, 1937; Underwood and Filmer, 1935) and Marston, Lines and McDonald in South Australia (Bull, Marston, Murnane and Lines, 1938; Lines, 1935, 1938; Marston, 1935, 1938; Marston and McDonald, 1938). The reason for the investigations, which led to the discovery, was the same in both instances but the avenue of approach differed somewhat.

Along the south coast of South Australia and Western Australia, areas occur in patches which have long been known by farmers to be unsuitable for raising sheep and cattle although they enjoy a rainfall of 20 to 40 inches and produce abundant herbage throughout a good portion of the year. Animals could be fattened on these pastures but if maintained upon them for more than 3 to 4 months they lost condition, became weak, lying about instead of roaming for food and, unless speedily removed to other pastures, wasted and died whilst, maybe, surrounded by plenty. Sheep which had not suffered too severely recovered rapidly after the transference.

In South Australia the soil of these unsound patches of country consists for the most part of windblown sand and shells, not yet consolidated. It is poorly mineralized and contains some 60 per cent. of $CaCO_3$.

In Western Australia, the areas where the disease has so far been studied are some 5 miles from the coast at an altitude of 500 feet; the soil is not calcareous but a loamy gravel formed by the weathering of old granitic rocks (Hosking and Burville, 1938).

Observations in Western Australia

For convenience of description, observations in Western Australia will be dealt with first. In 1933, after 5 years' study, Filmer (1933) published an account of a wasting disease of stock in the neighbourhood of Denmark, some 30 miles west of Albany. The areas in which the disease occurred were irregularly distributed.

The symptoms of the disease were merely those of starvation, namely, progressive emaciation and weakness and, in the later stages, an anaemia of the normocytic type. At autopsy little was seen beyond absence of fat, some fatty degeneration of the liver, and deposition of haemosiderin in liver, spleen and kidney. Otherwise the organs appeared normal. There was no evidence that an infective process was responsible for the disease. Moreover, infection seemed excluded, since sick animals, transferred to healthy herds or flocks on neighbouring sound country, did not prejudice the health of the latter but themselves recovered. Filmer suggested the name enzootic marasmus for the condition, and concluded that it was caused by some nutritive deficiency.

The administration of phosphate, iodide, copper and manganese was tried without producing any beneficial effect, so, in view of the general similarity between enzootic marasmus and bush sickness in New Zealand, which Aston (1928) had found amenable to treatment with large doses of iron, particularly in the form of the mineral limonite ($2Fe_2O_3,3H_2O$), pharmaceutical preparations of iron or limonite were given to sick animals. With the former, some success was obtained and the latter gave excellent results. Nevertheless, Filmer was not satisfied that it was iron which cured the beasts, because of the massive doses required and of the superiority of the crude mineral. He thought that it was more likely that enzootic marasmus was due to a deficiency of some other mineral which was present in small amounts in the iron preparation employed. He was joined by Underwood in 1931, and together they followed up this clue. The first step was the extraction with weak HCl of an active specimen of limonite, and the removal of the iron from the solution. This iron free extract was found to be a cure for enzootic marasmus as potent as the original limonite, whereas the iron separated from it was ineffectual (Filmer and Underwood, 1934).

The idea that iron was the curative agent having been disposed of, the next step was to fractionate the extract. On doing this, the active agent was found to be in the zinc group in which are also nickel, cobalt and manganese. Manganese had already been tried and found wanting; there remained only nickel, cobalt and zinc. Nickel was tried first. Two salts of supposed purity, $NiCl_2$ and NiO were administered to sick sheep. The latter was effective, the former not. In view of the difficulty of completely separating nickel and cobalt, it seemed possible that this specimen of NiO owed its curative power to traces of cobalt. Pure $CoCl_2$ was, therefore, given in daily doses of 1 mg. or less to sheep suffering from marasmus of moderate severity. The general condition of the

animals improved immediately and the blood returned to normal in one to two months (Underwood and Filmer, 1935).

Further experiments by Filmer and Underwood (1937) showed that sick sheep could be cured, and subsequently maintained in health on unsound farms for 14 months, with a daily dose of 0·1 mg. cobalt; cattle required from 0·3 to 1·0 mg. daily to maintain their health.

Observations in South Australia

The investigation of the wasting disease affecting sheep depastured on the unconsolidated dune soils along the coast of South Australia, which also led to the discovery that a small amount of cobalt was essential for sheep, was more difficult because, as ultimately transpired, the sheep suffered from a dual deficiency. In addition to the progressive weakness, emaciation and anaemia of enzootic marasmus described by Filmer, ataxia proceeding to complete inco-ordination of the movements of fore and hind quarters was frequently observed. These symptoms, which occurred more particularly in lambs and young sheep, were shown by Bull and his colleagues to be due to more or less extensive demyelination of some of the tracts in the spinal cord (Bull, Marston, Murnane and Lines, 1938). The lesions were similar to those first described by Stewart (1932) in the cord of sheep suffering from swayback in this country, the pathology of which has since been more intensively studied by Innes (1936).

A survey of the incidence and distribution of these nervous symptoms indicated that two maladies of different causation were frequently superimposed on one another in the coastal areas of South Australia. The ataxic disease was similar to, if not identical with, that described by Bennetts (1932, 1933) as occurring in a particular area in Western Australia where it was called gin-gin disease, and was shown later by Bennetts and Chapman (1937) to be due to a deficiency of copper. The acute wasting disease resembled bush sickness in New Zealand and enzootic marasmus as seen in Western Australia.

The epidemiology of coast disease did not suggest an infective origin and all efforts to discover an infective agent were fruitless. The possibility of the animals consuming some noxious weed which might grow particularly upon these dune areas and produce slow poisoning, an occurrence not unknown in Australia, was more difficult to exclude. However, a close scrutiny by a botanist of the herbage growing upon these areas failed to discover any poisonous plant. All the facts pointed to some nutritive deficiency of the pasture in the areas where the malady occurred and, as the animals grew well and fattened for some three or four months when first placed on these pastures, the deficiency was presumably a mineral one.

An outstanding peculiarity of the soil of these old dune formations is that they contain about 60 per cent. of $CaCO_3$ but less than one part per thousand of P_2O_5 (Thomas, 1938). This excess of lime is reflected in the composition of the pasture. It, therefore, seemed not unlikely that animals living upon it would suffer from phosphorus deficiency both on account of the small amount present in their food and the excessive overbalance with calcium. However, experiments undertaken to see whether administration of calcium diphosphate would mend matters entirely failed

to show any benefit therefrom (Lines, 1938) and, as the phosphorus in the blood of sheep inhabiting these unsound areas was found to be undiminished, phosphorus starvation seemed to be excluded. Administration of iron did not produce the same favourable results as had been found with bush sickness in New Zealand. Salts of copper and of manganese were also ineffectual.

By this stage of the investigation, the conviction was strong that lack of one of the trace elements was to blame. It was, therefore, decided to try the effect of a salt mixture compounded by Jansen (1933), which contains most of the elements that have been discovered in the bodies of animals in addition to those usually incorporated in salt mixtures for laboratory experiments. Amongst others are zinc, nickel, cobalt, manganese, aluminium, boron, molybdenum and copper in small amounts.

Jansen's salt mixture was administered daily to some sheep suffering from coast disease, the animals being maintained meantime on the unhealthy pasture (Marston, 1935; Lines, 1938). As a result, the treated ones speedily recovered their health and liveliness, and the anaemia steadily improved, whereas those not so treated deteriorated.

The question then arose as to which of the unusual constituents in Jansen's mixture was likely to be the effective one and, therefore, to be tried first. Since anaemia was a feature of coast disease, and cobalt in relatively large doses had been shown to stimulate erythropoiesis in rats and dogs,* it was given precedence. The first results in the laboratory from the administration of 1 mg. cobalt daily were dramatic; very sick sheep were speedily restored to health whereas their companions not so treated died (Lines, 1935). Further trials of cobalt salts with sick sheep in the field were, however, disappointing; some improvement in their condition occurred but no complete cure. The explanation of the discrepancy was not clear. Subsequently it was found that the gluten in the laboratory diet contributed copper also.

In a final, unexceptionable experiment by Marston and McDonald (1938) in which various combinations of zinc, iron, nickel, cobalt and manganese were administered to groups, each of 8 sheep, it emerged that only those animals recovered which had received copper along with cobalt. The control group without any treatment and all those treated with various combinations which did not include both copper and cobalt succumbed within a year (see Figure 1). These experiments of Marston, Lines and McDonald leave no room for doubt that coast disease occurring amongst sheep depastured on the old dune soils of South Australia is due to an insufficient amount of both cobalt and copper in their food.

Significance of the Australian Observations for Similar Diseases Elsewhere

The discoveries made in Western Australia and in South Australia were soon found to apply to bush sickness in New Zealand (Askew and Dixon, 1936; Askew, 1939), and pine in some parts of Scotland (Corner and Smith, 1938; Corner, 1939), and to a wasting disease of sheep in

* For reference to these observations see Marston's article, "Ruminant Nutrition," in the *Annual Review of Biochemistry*, 1939, **8**, 572, para. 2. Since then a great deal of work has been done to ascertain whether traces of cobalt are required by rats and dogs for blood formation. This is still uncertain.

Canada (Bowstead and Sackville, 1939), and are likely to find their application elsewhere, for instance, in Kenya to nakuruitis.

Valuable work has been done in New Zealand by Hopkirk and Grimmett (1938) and McNaught (1938) to ascertain the minimum amount of cobalt which must be contained in pasture to ensure the well being of stock. This would appear to be from 0·07 to 0·3 p.p.m. for sheep; for cattle as little as 0·04 p.p.m. was sufficient. Horses do not suffer from bush sickness. Methods for remedying defective pastures in New Zealand have been explored by Hopkirk and Grimmett (1938), Bonner, McNaught and Paul (1939), and Askew (1939). From their experimental trials it would appear that top dressing with half to one pound of a cobalt salt rendered these pastures capable of maintaining stock in health for at least one year.

Copper Starvation

The specific effects of copper deficiency have been referred to only incidentally but another interesting story could be told of the investigation by Bennetts (1932, 1933) of an ataxic disease of sheep occurring in localized areas of Western Australia. He and Chapman (1937) ultimately showed that this disease was caused by copper starvation and could be controlled by top dressing the land with a few lb. of $CuSO_4$ per acre. This nervous disease, which Bennett called enzootic ataxia, is evidently identical with renguerra in Peru, and swayback as seen in restricted areas in this country, Europe and South Africa.

Our Australian colleagues are to be congratulated in that, in a comparatively short time, they succeeded, not only in elucidating the causation of two mysterious diseases of stock which had baffled investigators elsewhere, but also in discovering the unexpected significance of traces of copper and cobalt in the nutrition of cattle and sheep.

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Further literature on the nutritive importance of cobalt will be found in an article by Underwood, "The Significance of Trace Elements in Nutrition," in *Nutrition Abstracts and Reviews*, 1940, **9**, 515, and also in an essay on ruminant nutrition by Marston in *Annual Review of Biochemistry*, 1939, **8**, 557. Several papers published since these reviews were written will be found in the *New Zealand Journal of Agriculture* and in the *New Zealand Journal of Science and Technology*.

Pining in Great Britain

Mr. W. Lyle Stewart (King's College, Newcastle upon Tyne)

The term pine or pining is probably derived from the Anglo-Saxon "pinian" to torment or waste away under pain, and is thus correctly and commonly applied to a number of diseases of sheep and cattle and also of man which are characterized by wasting. On the uplands bordering England and Scotland, however, the word has come to acquire a rather more specific meaning, and denotes a peculiar malady of hill sheep, the main features of which are anaemia and emaciation. This disease is of some antiquity, and the time honoured method of combating it is by moving the entire flock twice or thrice a year to a poorer but healthier pasture. This change, preferably to a heather moor, should be effected while the stock is still strong because, if delayed till many sheep are showing symptoms, it may be impossible to move the flock owing to their extremely weak and anaemic condition. For four or five days the sheep greatly relish a change to heather, but afterwards they tire of it, eat little, and appear eager to return home. Curiously enough the sheep look much worse just after such a change, and they lose weight, but the benefit, if somewhat delayed, is none the less substantial, and this system is commonly believed to have permitted sheep breeding on land where the entire stock would otherwise gradually die out. Apart from this disease, pining farms are generally healthy and may be more productive of wool and mutton than neighbouring areas where there is no pining.

My interest in pining commenced some years ago when, with Piercy, I made observations in the Cheviot Hills and recorded heavy trichostrongyle infestations in sheep dead from pining (Stewart and Piercy, 1935). We attributed death to parasitic disease, but were particularly careful not to exclude the prior operation of a nutritional factor. Later work on affected sheep, together with evidence from analyses of pasture and of the bones of pining sheep, suggested an association of internal parasites and malnutrition in the aetiological chain of events culminating in the clinical disease; this view was put forward by Dr. Ponsford and myself in 1936 (Stewart and Ponsford, 1936). Meanwhile there was a tendency on the part of a number of workers, both at home and abroad, to group pining with a number of specific deficiency diseases occurring in other parts of the world, such as bush sickness in New Zealand and coast disease in South Australia. Lack of iron and later of cobalt was incriminated as the specific deficiency. That this classification was little more than a shrewd guess is seen by the entire absence of confirmatory pathological data. It was based on analogy, and on the apparent

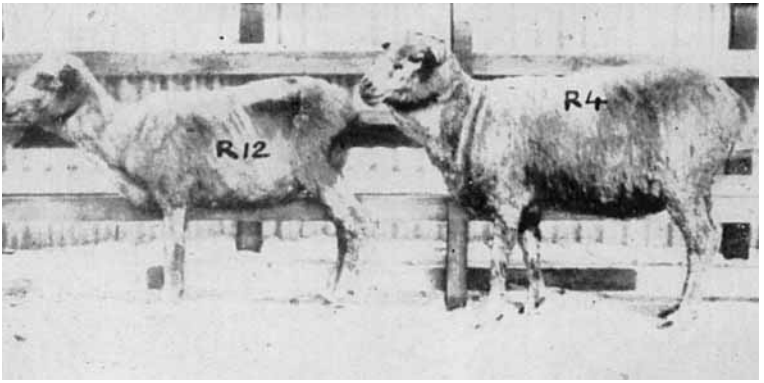

FIGURE 1. THE EFFICACY OF COBALT AND COPPER IN THE TREATMENT OF COAST DISEASE.

Ewes kept during 50 weeks on pasture known to produce coast disease. Both received thrice weekly 50 mg. iron and 2 mg. copper; R4 received in addition 2 mg. cobalt.

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success of experimental treatments often resting on superficial observations, and was too readily accepted as convincing proof of the true nature of pining.

In 1935 important results from cognate experimental work in Australia were published. Lines and Marston, and Underwood and Filmer discovered that a pasture deficiency of cobalt and copper played a prominent part in causing coast disease in sheep and cattle. Soon afterwards Askew and Dixon demonstrated that cobalt supplements were completely effective in controlling bush sickness (for literature see preceding paper by Martin, 1944).

Comprehensive field studies of pining were commenced in Northumberland in 1936 in an endeavour to obtain more precise information on the nature of pining, and with the further object of shedding light on the nutritional requirements of hill sheep. For a number of years, therefore, fundamental observations co-ordinating nutritional, parasitological and pathological features of pining have been made, and what follows is a condensed summary of the information that has been obtained.

Pasture Analysis. The main feature of the pasture analyses is that the pastures examined for causing pine are nutritionally poor, being equivalent in general feeding value to a fair sample of meadow hay. They are poor in lime and below the average in phosphorus. They are far from being deficient in iron though their iron content is generally lower than that of good grazing areas. The copper content is above the level of that in regions where copper deficiency has been established. The cobalt content is regarded as being on the low side, although not as low as in the cobalt deficient areas of New Zealand.

Incidence of Pining. Five groups of sheep were grazed continuously on a pasture liable to cause pine, fenced into five similar enclosures of about 40 acres each. Preventive treatments were devised and are set out in Table 1, where the incidence over 3 years of pining among the sheep on these plots is shown.

TABLE 1

INCIDENCE OF PINING AMONG SHEEP RECEIVING VARIOUS TREATMENTS

No. of plot	Treatment of plot	No. of sheep on plot	No. of sheep showing pine		
			Lambs	Hoggs	Ewes
1	Mineralized cake and anthelmintics ..	66	0	0	1
2	Anthelmintics	69	1	4	0
3	No treatment (controls)	71	3	7	11
4	Mineralized cake	70	0	1	2
5	No treatment beyond change of pasture twice yearly (controls) ..	63	15	7	13

Eighty-six per cent. of the deaths from pine occurred in the two control groups (plots 3 and 5). Perhaps the most surprising feature is the large number of deaths among the sheep on plot 5, which suggests that the periodic change of pasture twice a year not only proved of no benefit but was actually detrimental to the health of the sheep. The beneficial effect of regular anthelmintic treatment is also striking, confirming the

previous view, expressed by Stewart and Piercy (1935), that uncontrolled parasitic infestation is an important factor in the aetiology of pining. The value of supplementary feeding also is likewise readily obvious from the present work, but whether this was due to extra protein and energy intake or to the associated minerals or to both, is a question that the experiment was not designed to answer.

Liveweight Gains. The beneficial effects of the various treatments can also be assessed by the weight records from the various groups. All the sheep were weighed quarterly. The combination of supplementary feeding and anthelmintic treatment (plot 1) had the greatest effect upon the growth and condition of all the animals undergoing this treatment. There were no constant differences in favour of supplementary feeding alone (plot 4) over anthelmintic treatment (plot 2), but each gave considerably better results than were obtained in the control groups. The results are given in Table 2.

TABLE 2

MEAN LIVEWEIGHT INCREASES (lb.) OF EXPERIMENTAL SHEEP

Sheep		Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
Ewes	1st year	20·0 (7)	15·6 (8)	8·0 (7)	26·9 (8)	4·5 (8)
	2nd ,,	11·0 (7)	−4·4 (7)	2·2 (6)	0·0 (7)	2·8 (6)
Lambs	1st ,,	43·5 (15)	26·9 (15)	23·2 (13)	33·5 (13)	17·6 (14)
and	2nd ,,	13·1 (16)	10·1 (15)	7·4 (14)	8·3 (11)	4·9 (12)
hoggs						

Figures in brackets indicate number of animals on each plot.

Haematology. At each quarterly weighing all the ewes and lambs were bled, and blood chemical analyses were made according to standard methods. A great variety of determinations was made and the data on haemoglobin and red cell content are summarized in Table 3.

TABLE 3

BLOOD ANALYSES OF PINING AND NORMAL HILL SHEEP

	Pining			Normal		
	Mean	Range	No. of animals	Mean	Range	No. of animals
Haemoglobin g. per 100 ml. ..	7·2	3·2 to 11·3	40	10·6	7·6 to 14·9	107
Haematocrit cell vol. per cent.	19·5	9·0 to 30·5	40	29·0	20·5 to 38·0	526
Red cells millions per cmm.	8·0	2·5 to 12·0	19	12·4	7·1 to 14·5	45

In pining, therefore, there was a reduction in the number of red cells in the blood and, in some cases, the anaemia was extreme. In other cases, the blood presented an almost normal picture while clinical signs of pining were marked. The haematocrit values and red cell counts closely followed the haemoglobin values. Haematocrit values below 23 per cent.

and red cell counts below 8 millions were indicative of anaemia although, as with the haemoglobin values, the distinction between pining and non-pining sheep was not clear cut. The anaemia was not hypo- or hyperchromic.

Helminthology. The results of helminthological studies are given in Table 4. The post mortem findings on 65 pining sheep confirm the previous observations of Stewart and Piercy (1935) and supply unquestionable evidence of the important association of worms with pining. The worm burdens of affected lambs were much lower than those found in ewes, and there were also many more deaths from pining among the older sheep. If pining resulted solely from parasitic disease the heaviest mortality would undoubtedly occur among lambs, which have little or

TABLE 4

WORM COUNTS IN SHEEP DYING FROM PINING AND FROM OTHER CAUSES, 1936–1939

Plots	Deaths from pining	Average worm counts*	Deaths from other causes	Average worm counts*
1. Mineralized cake and anthelmintic (66 sheep)	Lambs 0	—	Lambs 0	—
	Hoggs 0	—	Hoggs 0	—
	Ewes 1	—	Ewes 3	—
	Total 1		Total 3	
2. Anthelmintic (69 sheep)	Lambs 1	5000	Lambs 0	—
	Hoggs 4	42,000 (13,000 to 78,000)	Hoggs 2	—
	Ewes 0	—	Ewes 2	300
	Total 5		Total 4	
3. Control, no treatment (71 sheep)	Lambs 3	27,000 (15,000 to 48,000)	Lambs 0	—
	Hoggs 7	31,000 (13,000 to 60,000)	Hoggs 3	—
	Ewes 11	62,000 (5000 to 95,000)	Ewes 2	21,500
	Total 21		Total 5	
4. Mineralized cake (70 sheep)	Lambs 0	—	Lambs 0	—
	Hoggs 1	41,000	Hoggs 2	5000 (in one animal)
	Ewes 2	33,000 (23,000 to 43,000)	Ewes 7	10,000 (3000 to 29,000)
	Total 3		Total 9	
5. Control, no treatment, change of pasture (63 sheep)	Lambs 15	15,000 (5000 to 44,000)	Lambs 1	—
	Hoggs 7	24,000 (10,000 to 41,000)	Hoggs 0	—
	Ewes 13	37,000 (5000 to 77,000)	Ewes 2	3000 (600 to 6000)
	Total 35		Total 3	

* Figures in brackets denote range.

no immunity; the facts elicited go to prove that pining is primarily due to an impairment of the powers of resistance normally acquired by healthy adult sheep.

The field experiments have since been extended to find what factor or factors would prevent the disease. The results indicate that a complete mineral supplement enables sheep to maintain condition and gain in weight, those receiving anthelmintic treatment in addition making an even more satisfactory gain. Sheep receiving no treatment or anthelmintics alone have barely maintained their original weights (see Table 5).

TABLE 5

EFFECT OF DIFFERENT TREATMENTS ON LIVEWEIGHT OF SHEEP

	Anthelmintic (42 sheep)	Minerals (42 sheep)	Combined treatment (42 sheep)	No treatment (42 sheep)
Average liveweight, lb. Sept. 1939	80	76	75	74
,, ,, Aug. 1940	80	81	82	73
Change	0	+5	+7	−1

These field studies on pining have been exceedingly useful and have provided an opportunity of gaining, at first hand, information as to methods of sheep farming and sheep nutrition, which would otherwise have been very difficult for a scientist to obtain. The general plane of nutrition on many hill farms is unnecessarily low, and production of wool and mutton correspondingly reduced. Grading up is required and is feasible. I am opposed to pampering hill sheep by feeding them with protein or energy foods, even if available, but nothing but good can result from making up the known and perhaps the unknown mineral deficiencies and, at the same time, controlling the parasitic factor by regular anthelmintic treatment. Probably one of the best ways of doing this is to allow the sheep free access to a complete mineral mixture, preferably fed in cube form. I am not in favour of using salt licks; they are much too expensive and mostly contain common salt. The anthelmintic of choice is undoubtedly phenothiazine.

Finally, some figures may be included obtained from a recent experiment on a pining farm. The results have not yet been statistically analysed and only the arithmetical means can as yet be supplied. It is to be hoped that they will not prove misleading.

No treatment (20 lambs)	Lambs gained an average of 27·0 lb.
Phenothiazine (20 lambs)	,, ,, ,, ,, ,, 31·5 ,,
Trace elements (20 lambs)	,, ,, ,, ,, ,, 30·7 ,,
Combined phenothiazine and trace elements (20 lambs)	,, ,, ,, ,, ,, 35·7 ,,

These groups of lambs were properly randomized and, throughout the test, all groups were grazed and managed under identical conditions. The effect of treatment is readily seen.

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Discussion

Dr. E. L. Taylor (Veterinary Laboratory, Ministry of Agriculture, Weybridge, Surrey), opener: The discussion on this very interesting series of new observations must be opened by calling attention to the effect of nutritional deficiencies on infectious disease, particularly on disease caused by parasitic helminths.

It is well known to helminthologists that, as young animals grow to maturity, it is normal for them to develop towards most species of parasitic worms, a relatively strong resistance which is sufficient to maintain them in health in all ordinary conditions of grazing. This resistance is only relative, however, and may break down under a variety of stresses, one of the most important of which is nutritional insufficiency.

It has been shown, for instance, that sheep which have developed a resistance to the stomach worm, *Haemonchus contortus*, can be made susceptible if placed on an inadequate diet, and can throw the infestation off again when a full diet is restored. Resistant adult dogs become susceptible to hookworm infection in similar conditions of inadequate diet, and lose infection as soon as they return to a full diet.

It has been observed by several workers that the resistance of the host to helminthiasis and also to diseases caused by microscopic parasites is not affected by nutritional insufficiency unless this is sufficiently severe to affect the general condition of the animal. These workers have concluded that diet cannot play any important practical part in the development of infectious disease. A similar conclusion has been reached by epidemiologists, who have expressed the opinion that only dietary defects of the most serious character such as gross lack of vitamins or frank starvation among large sections of a population could be of importance in determining the incidence or severity of epidemic disease. These are conditions which are unlikely to exist among human populations, at least in such countries as ours.

Grazing animals are, however, less favourably placed and, for months at a time, are forced to exist on a starvation diet that may sometimes be barely sufficient to keep them alive. During the winter months sheep may actually lose anything up to a quarter of their weight, and store cattle are often kept only just alive on nothing but the poorest of hay and straw.

This nutritional stress comes at a period in which the young animal ought to be establishing its immunity and throwing off the parasites acquired during the summer; as a result the parasites remain, or increase in numbers if the animals are still grazing, and a considerable loss in weight occurs. Anything which interferes with the establishment of the resistance of young grazing animals to the parasitic worms present in all ordinary pastures is of great economic importance; the establishment of that resistance is imperative if health is to be maintained and growth continue.

Effects similar to those of rank starvation have been produced experimentally, through vitamin deficiency and iron deficiency. There is now evidence that trace element deficiency may act in the same way, and it seems highly probable that, in addition to the various manifestations of disease directly attributable to a deficiency of trace elements, further

loss among grazing animals may be caused through parasitic infestation resulting from the deficiency.

Dr. G. Bourne (Physiological Laboratory, Oxford): Filmer (1933) and Filmer and Underwood (1937) mention that in cobalt deficiency lactation is suppressed and Graafian follicles fail to ripen. These findings suggest an effect of cobalt deficiency on the pituitary gland. In Filmer's experience the activity of cobalt was increased by the presence of traces of nickel.

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Dr. H. H. Green (Veterinary Laboratory, Ministry of Agriculture, Weybridge, Surrey): Depression of lactation and interference with ovulation are not characteristic only of cobalt deficiency but are produced also by many other types of deficiency. There is no evidence of specific association between cobalt deficiency and pituitary function. Filmer's data do not appear to justify any conclusions with regard to nickel.

Afternoon Session: Chairman, Dr. C. R. Harington

Fluorine in Human Nutrition

Biochemical Aspects

Dr. M. M. Murray (Department of Physiology, Bedford College for Women, University of London)

Fluorine is the first element of the halogen group, it is the most electronegative of all elements, is very reactive and hence does not occur free in nature. It occurs often associated with calcium, for example as fluorspar or calcium fluoride, or as fluorapatite, a form of calcium phosphate in which fluorine replaces the hydroxy groups of the hydroxyapatite which is the main constituent of vertebrate skeletal structures. Fluorine occurs in all rock phosphates. These minerals together with cryolite, which is sodium aluminium fluoride, and volcanic deposits are some of the chief, but not the sole, sources of the fluorine occurring in drinking water, and causing chronic endemic fluorosis, the most easily recognized sign of which is mottling of the teeth.

Mottled enamel is characterized by the presence of opaque white patches or bands irregularly distributed over the tooth. Sometimes the whole tooth has this opaque white appearance. The characteristic mahogany brown staining may or may not be present; it is a secondary phenomenon, probably due to infiltration. The nature of the staining is

at present unproven. A mottled tooth may be of good structure or may be irregularly formed and pitted and of poor structure.

Fluorspar, which is used as a flux and for glass etching, and cryolite, which is an important source of aluminium, are in their uses the chief causes of industrial fluorosis, in which the most marked symptom is osteosclerosis.

Fluorine occurs to a small extent in all the apatite structures of the vertebrate body, namely, in bones and teeth. Table 1 shows values for the percentage content of fluorine in normal and fluorotic bones and teeth.

TABLE 1

FLUORINE CONTENT OF HUMAN BONES AND TEETH

Tissue	Percentage F
Bones	
Normal, age 1 year	0·03 (Glock, Mowater and Murray, 1941)
,, ,, 40 ,,	0·20 (Glock, Mowater and Murray, 1941)
Cryolite worker with severe osteosclerosis	1·00 (Roholm, 1937)
Teeth (enamel)	
Sound (Minnesota)	0·0111 (Armstrong and Brekhus, 1938)
Carious ,,	0·0060 (Armstrong and Brekhus, 1938)
Sound (Tristan da Cunha)	0·0140 (Sognnaes and Armstrong, 1941)
Severely mottled (Maldon)	0·0320

Nutritionally fluorine holds a curious position even as a trace element. Until a few years ago it was thought harmful to man and animals in all amounts in the diet though difficult to avoid entirely. Crucial experiments have not been made to ascertain whether it is an essential element or not.

Since the classical work of Black and McKay (1916), mottled teeth have been considered relatively resistant to dental caries but it was, nevertheless, thought advisable in the U.S.A. to change any water supply which contained more fluorine than 1 p.p.m. Not many waters so far analysed in this country contain more than this amount; some results of such analyses are given in Table 2. Maldon, in Essex, England's

TABLE 2

FLUORINE CONTENT OF SOME ENGLISH WATER SUPPLIES

Locality	Fluorine content p.p.m.
Maldon, Essex	5
Oxford City	0
Oxfordshire — Hook Norton	0·45
Oxfordshire — Launton	0·85
Somerset, Timberscombe	0·45
London — Thames	0·10
London — New River	0·30
London — River Lee	0·15

show place for mottled teeth, used to use a well water with 5 p.p.m.; this is believed to be now diluted with Southend water. This mixing

procedure is to be commended for either lowering or raising the fluorine content to what is now believed to be a beneficial value, that is to, say, one between 0·5 and 0·3 p.p.m.

Epidemiological studies by Dean (1938) and others in America have proved statistically that teeth with mottled enamel are relatively immune to dental caries even when the fluorine in the drinking water is less than 1 p.p.m. It has recently been shown (Spira, 1942; Wilson, 1941) that in this country mottling of a mild degree is of frequent occurrence, that it can be related to small concentrations of fluorine in the water (Murray and Wilson, 1942) and that, in certain areas investigated, there was in children under 14 a negative correlation between mottling and incidence of caries (see Table 3).

TABLE 3

INCIDENCE OF MOTTLING AND CARIES IN LONDON
(Murray and Wilson, 1942)

No. of children, ♀ and ♂, 10 to 15 years	Degree of mottling	No. of teeth carious	No. of teeth carious per head
167	none	688	4·1
83	very mild	89	
54	mild	15	
7	moderate	6	
6	severe	7	
Total 150	All grades	Total 117	0·8

In America Armstrong and Brekhus (1938) found more fluorine in sound than in carious teeth. It is interesting that the teeth, highly immune to caries, of the inhabitants of the island of Tristan da Cunha, which have been quoted as a test case for most theories of dental caries, have been described as showing threshold mottling, and found to contain an amount of fluorine greater than Armstrong's figure for sound teeth (Sognnaes and Armstrong, 1941).

Mottling can only occur by the action of fluorine during development; hence, permanent teeth are more frequently affected than temporary teeth, which, however, can also be affected if the mother's intake of fluorine is high enough to cause some fluorine to pass the placental barrier, and to pass into the milk. Fluorine can affect the bones at all ages; an adult industrial worker exposed to fluorine does not develop mottled teeth but gets osteosclerosis, although the offspring of female cryolite workers have been known to have mottled temporary teeth.

Little is known about the action of fluorine; the concentration at which it is effective suggests a catalytic effect or an inhibitory action on an enzyme system. It would seem possible that, in very low concentrations, fluorine favours proper calcification, though sodium fluoride in concentrations between M/10,000 and M/100,000 inhibits calcification in bone slices (Gutman, Warrick and Gutman, 1942; Robison and Rosenheim, 1934). When female rats were given small amounts of sodium fluoride,

the process of calcification in the foetuses and new born offspring showed definite disorganization (Glock, 1940).

It is possible that some localities like Tristan da Cunha provide in the drinking water and in food just the right amount of fluorine and that this, together with an adequacy of the other factors necessary for good calcification, promotes the proper development of the skeleton and the formation of teeth of good structure with high resistance to caries.

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See also a review: Volker, J. F. and Bibby, B. G. (1941). *Medicine, Baltimore*, **20**, 211.

Clinical Appreciation of Fluorine Distribution

Dr. D. C. Wilson (10 Parks Road, Oxford)

While the degree of mottling and staining of the teeth is proportionate to the amount of fluorine present in soil and water, the character of the enamel, whether smooth or pitted, and the regularity of the dentition, is determined by the state of nutrition of the individual. Well formed enamel is seen only where the state of nutrition is good. Comparative dietary surveys and nutritional examinations show that these correlations exist whether the dental comparison is made in India between poor rice eaters in the south and certain well nourished agricultural tribes of the north, or in England between the well fed children of farmers in Weardale, Durham, and the ill nourished offspring of china clay workers in Cornwall.

The state of nutrition influences in the same way the incidence and degree of skeletal lesions in chronic endemic fluorosis with similar fluorine content in water. Severe spine lesions are seen frequently amongst poor villagers in south India while few are found among more prosperous communities in the north, though the fluorine content of the water is similar in the two localities. Pundit and his colleagues in careful comparative dietary surveys in two adjacent south Indian villages found that, in both, the degree of mottling of enamel was related to the amount of fluorine in the water, but that the bone lesions were related to the state of nutrition, being considerably higher in the poorer village with the more deficient diet, even though the fluorine content of the water was slightly less (Pundit, Raghavachari, Rao and Krishnamurti, 1940). The occurrence of "round backs" in Oxfordshire children with dental fluorosis will be described by Dr. Kemp. From the clinical standpoint, increased emphasis on posture during physical training has been suggested

as useful early corrective treatment for children whose nutrition is already improved through the addition of school milk and dinners.

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Radiological Investigations

Dr. F. H. Kemp (Radcliffe Infirmary, Oxford)

The radiological investigations to be described originated in the observation of the frequency of "round back" among children and adults in areas where mottled enamel was prevalent.

Endemic dental fluorosis occurs in many parts of the world but cases of chronic fluorine poisoning with skeletal changes seem to be comparatively rare. Shortt and his colleagues reported a study of 10 cases in the Madras Presidency (Shortt, McRobert, Barnard and Nayar, 1937). At about 30 years of age the first signs of poisoning appeared, evidenced by a general tingling in the limbs and body. Pain and stiffness next appeared, especially in the lumbar spine. The stiffness increased until the entire spine, including the cervical region, behaved like a continuous column of bone. There was stiffness of other joints and ossification of the periarticular tissues, tendinous insertions and interosseous fasciae. The ribs became fixed and the breathing entirely abdominal. By the time the condition was fully developed the patient was about forty years old; there was much cachexia, and death ensued from intercurrent disease. There were minor variations, and all cases in a village were not of the same degree of severity. The amount of fluorine in the drinking water responsible for these cases was between 3 and 4 p.p.m. Radiograms of these Madras cases showed changes similar to those described in cryolite workers by Flemming Møller and Gudjonsson (1932).

Changes similar to those described by Shortt have been reported from South Africa by Ockerse (1941), who found 15 areas in the Pretoria district where mottled enamel is endemic.

As far as can be ascertained, skeletal changes associated with mottled enamel in human beings have not been reported in this country or in America (Hodges, Fareed, Ruggy and Chudnoff, 1941).

We decided to examine a group of children to see whether any disturbances of natural growth could be determined, since a toxic factor operating at a very low concentration would be most likely to produce changes during the most active phases of bone growth, in situations most subject to stresses and strains. The spine was selected for examination because, towards puberty, growth is centred in relatively thin layers of tissue in the end plates of the bodies of the vertebrae which, with every movement, are subjected to considerable changes of pressure (Kemp, Murray and Wilson, 1942). We have found that interference with the normal course of ossification of the spines of children is relatively frequent, though not often seen in hospital practice, and that dental fluorosis is commonly associated with such changes. There is no evidence that fluorine is solely responsible for these malformations of the spine, but we

believe that fluorine in the water supply may influence the development of such defects, especially if associated with defective nutrition.

Signs of similar developmental disturbances and of early *spondylosis deformans* were found among many young adults, in some of whom dental fluorosis was also noted.

These findings are in accord with clinical experience which suggests that *spondylosis deformans* or spinal rheumatism is the result of the progressive degeneration of malformations laid down in youth.

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Industrial Fluorosis of Animals in England

Mr. F. Blakemore (Institute of Animal Pathology, Milton Road, Cambridge)

Industrial fluorosis has been reported in many parts of the world, the industries mainly concerned being those producing superphosphate, hydrochloric acid, bricks, aluminium, glass and enamel. In this country it has been studied in stock kept on pastures near certain brickworks. The source of the fluorine was determined from examination of the flue gases, detection of fluorine in the atmosphere of the district, and evidence of surface contamination of plants in the area. It would seem that large collections of kilns may contaminate pastures to a distance of one mile on the windward side to such an extent as to give rise to symptoms in cattle grazing on them. An examination of the stock on affected farms revealed that the severity of the symptoms depended upon the degree to which the herbage was contaminated, and the severely affected districts merged gradually into the normal areas. A great difference in the susceptibility of different farm animals was apparent, cattle being the most prone to develop symptoms.

The chief symptom shown by severely affected cattle was lameness. There was an increase in the diameter of the metacarpals, metatarsals and phalanges, and palpation of the bones caused considerable pain. Mottling of the permanent incisors was constantly present in cattle reared in the district. Post mortem examinations showed that the changes were limited to the skeleton. The bones were enlarged, their specific gravity reduced and their fragility increased. The main histological features were extensive atrophy of the bony tissue and the presence of excessive amounts of osteoid. The fluorine content of the bones varied between 6000 and 16,000 p.p.m.

Estimation of the amount of fluorine in the urine proved to be a most valuable means of assessing the danger of fluorine poisoning on affected forms. An investigation was made to determine the rate of excretion of fluorine from the bones in cattle removed from the district. It was found that the bone content fell rapidly during the first 90 days, but that

this rapid rate of elimination was not maintained and figures for the following 150 days remained fairly constant.

In view of the high susceptibility of cattle to fluorosis, examinations were made in south east Lancashire to ascertain whether cattle grazing within the Manchester smoke belt showed signs of fluorine poisoning, but clinical observations did not reveal any signs of the disease and the rate of urinary excretion of fluorine was normal in all the cattle examined. Analysis of hay samples also failed to show any serious contamination.

Discussion

Mr. C. N. Bromehead (H.M. Geological Survey, Exhibition Road, London, S.W.7), opener: The previous speakers have described some of the effects of an excessive intake of fluorine. In order to control intake, of which there is an optimum, it is essential to know the sources from which fluorine reaches the human beings or animals. It is universally agreed that, except for acute cases of workers handling materials known to contain fluorine, such as fluorspar or cryolite, the immediate source is drinking water. In this country almost all underground water supplies from wells and borings are derived from sedimentary rocks. When Dr. D. C. Wilson approached the Geological Survey on the question of fluorine in British waters, and the Director instructed me to attend to the problem, I was, apart from having heard of Maldon, completely ignorant of the subject; as far as I know my ignorance was shared by every geologist. Even in the United States, where fluorosis has received much attention, one geologist recently wrote that the distribution of fluorine in sedimentary rocks was so erratic that he could not discuss it (Nichols, 1939); another has published fluorine estimations for 78 rocks, of which only 6 are sedimentary and half of those limestones, the least likely type to be important (Shepherd, 1940).

Two methods of approach to the problem suggested themselves. After studying such known facts and speculations as appeared relevant, I could point out to Dr. Wilson, for the investigation of the incidence of fluorosis, certain areas which seemed likely to give a positive result. At the same time Dr. Wilson could investigate any areas convenient to her, and a study of the distribution of positive and negative results might indicate unexpected sources of fluorine. Both methods have been profitable. While many years' work may yet be required to give an adequate picture of the distribution of fluorosis in Britain, some useful indications are already visible; the areas where fluorosis is noticeably common are shown in Figure 1. Area 1 is Upper Weardale. It was an obvious step to investigate those areas where fluorspar is abundant, such as parts of Derbyshire, Westmoreland and Weardale. Striking results did not seem likely; calcium fluoride has a very low solubility and it was likely that all available fluorine was in that form. Derbyshire did not yield much result, but the fluorspar district of Weardale was clearly marked out. The difference is almost certainly a question of the source of the water supply in use; in Weardale this is largely abandoned and flooded mines in which the spar occurs.

Fluor-apatite (fluo-phosphate of lime) is considerably more soluble; this mineral is abundant in the china stone and china clay area of Cornwall. Here a noticeable concentration of fluorosis was found (Area 7).

Almost 50 years ago Carnot (1893) showed that fossil bones consisting mainly of calcium phosphate tend to take up fluorine, steadily approaching the composition of apatite with increasing age. Areas where phosphatic rocks are abundant, therefore, deserved immediate attention. My

FIGURE 1. AREAS IN ENGLAND AND WALES WHERE FLUOROSIS IS COMMON.

colleague, Dr. Oakley, has been preparing an account of the British sources of phosphate; he suggested the Spilsby area of Lincolnshire, where Lower Cretaceous phosphates occur and the local wells often, particularly in the village of Partney, get their water from the phosphate bed. This is Area 2 on the map. The Cambridge phosphate district is Area 4; the Maldon district of Essex, the classic area for fluorosis as shown by Ainsworth (1933), is Area 3. The high fluorine content of the well waters

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in the Maldon area is almost certainly due to the coincidence of a not abnormally high concentration of apatite in the Thanet Sand, from which the water is derived, with an unusually high content of sodium chloride; sodium fluoride is readily soluble and easily parts with its fluorine.

Dr. Wilson's most intensive survey has been around Oxford. The results are strongly suggestive of sources for the fluorine in the Jurassic Clays, and the Kimmeridge, Oxford and Lias Clays in descending order of stratification. The high fluorine content of parts of one of these formations in certain areas was already known. In Somerset there is a tendency towards concentration of fluorosis on the Lower Lias; it is, however, not so strictly confined to that formation as is the teart or molybdenum disease which will be dealt with in the next paper (Ferguson, 1944).

There remains Area 6 in Glamorganshire; here fluorosis is generally distributed. The area where the surface formation and/or the source of the water supply is the Coal Measures is as strongly marked as in the south where it is the Lower Lias. This result is puzzling: I should not have expected an appreciable fluorine content in such non-marine sediments as the Coal Measures. It is, however, noticeable that according to a report just published by the Fuel Research Board (1942) some South Wales coals are exceptionally high in phosphorus. As much as 4 per cent. of the ash has been recorded: in other coalfields 1 per cent. is exceptional. Details of the distribution are not yet available. It has already been mentioned that phosphates are normally fluorine bearing; there may, therefore, be an appreciable amount of fluorine present in parts of the Coal Measures of South Wales, possibly derived from the Lower Lias which formerly overlay them. A small amount of fluorine would be likely to produce noticeable results among the population of what was recently a depressed area where there would be much malnutrition.

In Mr. Blakemore's experiment the choice of the Manchester district for a test of the effects of factory smoke was unfortunate, except as a control; there the bricks are manufactured from deposits of non-marine origin which would be unlikely to contain fluorine. It might be desirable to test fumes from those brick works where the principal marine clays are used, such as Devizes for the Gault, Shotover for the Kimmeridge, east of Bicester for the Oxford, and Stroud to Cheltenham for the Lias.

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Dr. H. A. Krebs (Department of Biochemistry, University of Sheffield): Eight out of twenty-two factory workers employed in the manufacture of fluorine compounds in Sheffield showed radiological signs of osteosclerosis. The average daily urinary excretion of fluoride of workers exposed to hydrofluoric acid fumes was 15·1 mg. Those exposed to aluminium fluoride dust and to sodium, ammonium and magnesium fluorides excreted

7·2 and 9·6 mg., respectively, while the urinary output of normal controls was 1·8 mg.

Dr. H. M. Sinclair (Oxford Nutrition Survey, 10 Parks Road, Oxford): What evidence is there that fluoride intoxication caused the spondylosis in *all* cases shown by Dr. Wilson and Dr. Kemp? For example, the radiographs of the spines of the two students showed Schmorl's nodes, but no osteosclerosis or osteophytes, and neither apparently had an abnormal intake of fluorine, or dental fluorosis. I ask because I have Schmorl's nodes in my lumbar spine and also osteophytic exostoses elsewhere, but neither condition is due to fluorine intoxication.

Dr. J. D. King (Nutrition Laboratory, National Institute for Medical Research, Mill Hill, London, N.W.7): In Ipswich, Maldon, Burnham and certain parts of Oxfordshire more than half of the carious teeth are also mottled, while less than half of the caries free teeth show signs of mottling. The figures for incidence of caries in 1234 permanent premolar teeth in Ipswich are as follows:

Of the teeth with no mottling				7	per cent.	were	carious		
,,	,,	,,	,, grade	1	,, 17	,,	,,	,,	,,
,,	,,	,,	,,	2	,, 21	,,	,,	,,	,,
,,	,,	,,	,,	3 to 6	,, 33	,,	,,	,,	,,

These findings do not seem to support the views of Dr. Murray and Dr. Wilson.

Professor A. C. Frazer (Department of Pharmacology, Medical School, Hospitals Centre, Birmingham, 15): Is there a turn over of fluoride in bones comparable with the normal turn over of calcium? Parathyroid hormone increases the turn over of calcium and is used in chronic lead poisoning to facilitate removal of lead from the bones. Is the parathyroid hormone effective in removing fluoride from bones in fluorosis?

Dr. M. M. Murray:

In reply to Dr. J. D. King: The conception that mottling is associated with increased resistance to caries is based on a considerable amount of evidence put forward by Dean (1938) and by others but little work has been done in this country and further investigation is needed.

In reply to Professor Frazer: Even in adult animals fluorine can accumulate in or be eliminated from bones. Parathyroid hormone increases generally the rate of turn over in bone and would presumably increase the rate of elimination of fluorine.

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"Teart" of Somerset: A Molybdenosis of Farm Animals

Mr. W. S. Ferguson (Jealott's Hill Research Station, Bracknell, Berks.)

In Central Somerset there is a considerable area of pasture, probably at least 20,000 acres, which causes scour in grazing cattle. Smaller areas are found in north Somerset, Gloucester and Warwick. Such land is known in Somerset as "teart" and it causes considerable inconvenience and loss to the local farmers who are mainly engaged in dairying.

The degree of teartness varies from field to field and from district to

district. Some fields can cause scouring throughout the grazing season whereas, with others, the trouble is only noticed in the late summer or autumn. Scouring is most marked in the periods of flush growth in spring and late summer, but it is generally far worse in the late summer. As the herbage matures the scouring effect becomes less, but even hay may cause quite considerable scouring. Teart pasture is usually cut for hay and then left until the winter, when the frost kills most of the grass and renders it safe for cattle.

Only ruminants, particularly cows in milk and young stock, are affected by such pasture; strong, fattening stock, though they may not scour badly, are usually unthrifty and do not grow well. Scouring may start within 24 hours of putting the cattle in the pasture. The dung becomes extremely loose and watery, is yellow green in colour, and bubbly, and has a foul smell. The animals soon become filthy, their coats stare and they lose condition rapidly. Red Devon cattle turn a dirty yellow, and black beasts go rusty in colour. If left too long on teart land cattle may die or suffer permanent injury. The rapidity with which the scouring stops when the cattle are removed to non-teart pasture is very characteristic. Sheep are not so seriously affected but they become loose and dirty.

The trouble has been known for over 100 years and numerous experiments have been made to trace the cause. These, though unsuccessful, eliminated many of the most likely causes such as bacterial infection, parasites, toxic weeds and faulty water supply. They also showed that teart and non-teart pastures could be found in adjoining fields and that teartness is closely associated with the Lower Lias.

We decided to investigate the problem from the point of view of the minor elements and to look in the herbage for any deficiency of an essential element or presence of a toxic one which might cause the disturbance. We, therefore, examined spectrographically a large number of grass samples from teart and non-teart pastures and found that the only marked and consistent difference between them was the molybdenum content. The non-teart pastures contained usually less molybdenum than 5 p.p.m. in the dry matter, whereas in teart pastures values up to 100 p.p.m. were obtained. Moreover, the molybdenum content was directly related to the degree of teartness. Young herbage contained more molybdenum than old herbage. At one farm, where we were able to test, by grazing, over a period of three years, the teartness of the pasture, the average molybdenum content of the pasture was 40 p.p.m. in April when definite but not too severe scouring occurred, and 75 p.p.m. in September when scouring was very severe. The hay made from teart pasture, and almost dead winter grass, contained appreciable quantities of molybdenum and might have been expected to cause trouble, but it was found that the molybdenum in them was less soluble in water than that in young grass. Thus the percentage of molybdenum soluble in water was, in young grass, 70 to 80, in hay 40, and in dead winter herbage 10. It seems, therefore, that, although the teartness of the herbage is roughly related to its total molybdenum content, it is more closely related to its content of water soluble molybdenum.

The next step was to see if administration of molybdenum salts would cause scouring in cattle. Numerous tests were made which proved

conclusively that sodium and ammonium molybdate could cause severe scouring apparently identical with that seen in Somerset. They also showed that weak animals were more susceptible than strong ones, that the animals living in the teart area were more susceptible than those living outside the area, and that the effect was much more marked when the cattle were on pasture than when on winter rations. Scouring in cows on non-teart Somerset pasture was caused in 5 to 10 days by the daily ingestion of 1·7 g. sodium molybdate containing 0·68 g. molybdenum. This quantity of molybdenum would be found in a normal daily ration of teart pasture containing 50 p.p.m. molybdenum in the dry matter. About three times this amount was required to make strong cows at Jealott's Hill scour to the same extent.

In order to test whether molybdenum could be taken up by the pasture from the soil, an acre of sound pasture at Jealott's Hill was dressed with 20 lb. sodium molybdate during the winter. During the whole of the following summer cattle grazing on this pasture scoured very severely, and could only be left on the pasture for short periods.

All this evidence seemed sufficient to prove that the molybdenum contained in teart herbage is the cause of the scouring; the next step was to look for an antidote which could be given to the animal and for a means of preventing the absorption by pasture plants of molybdenum from the soil. It is not proposed to say anything on the second of these problems, but Dr. Lewis has discovered a number of interesting facts which it is hoped he will deal with in the discussion.

When we were considering the question of an antidote, a paper was published by Brouwer and his colleagues (Brouwer, Frens, Reitsma and Kalisvaart, 1938) describing a type of scouring, apparently very similar to teart scouring, which occurs on a reclaimed Polder. These workers suggested that the disease is caused by a variety of factors, one being a deficiency of copper. We therefore examined again the copper content of the teart and non-teart herbage, but no difference was found, the copper contents varying between 11 and 18 p.p.m. in the dry matter. These figures are about double those quoted by Brouwer and it seemed clear that teartness was not due to a deficiency of copper in the herbage.

Brouwer was able to prevent or cure the scouring trouble observed by giving small quantities of copper sulphate and cobalt nitrate and we therefore tried the effect of these salts and also of ferric chloride on cattle scouring in Somerset. From many preliminary tests it appeared that a daily dose of 2 g. copper sulphate was effective in stopping the scouring in dairy cows but that the addition of cobalt nitrate had no apparent effect. Ferric chloride was definitely less effective. These tests were promising enough to justify a large scale trial. Twelve farmers co-operated and the procedure was to put the test animals on teart pasture until they were scouring badly and then, whilst still on the herbage, to give them copper sulphate, the state of the dung being then watched. The results were highly conclusive. Fifty-nine animals, 49 dairy cows and 10 heifers were cured of scouring within a few days by administration of 2 g. copper sulphate daily. This trial was completed in 1939, and since then many farmers have given copper sulphate to their cattle whenever they showed signs of scouring on teart herbage, and have all reported satisfactory cures. By use of copper sulphate, teart pastures

can now be grazed throughout the season and thus be utilized much more efficiently than under the old system of management.

We were naturally somewhat concerned as to the effect of continued daily ingestion of copper sulphate, although the scanty data available in the literature suggested that the proposed dosage of 2 g. daily was unlikely to cause any disturbance. To test this, 6 dairy cows were given 2 g. of copper sulphate daily for 18 weeks; they remained in perfect health throughout the period. The average blood content of copper rose from 0·094 mg. per 100 ml. to 0·235 mg., an increase of 133 per cent., but the highest individual value, 0·264 mg., is similar to values quoted for normal oxen. When the dose was stopped the values fell to their initial level. In practice, the continuous administration of copper sulphate should not be necessary for more than 6 weeks and it is, therefore, unlikely to cause any physiological disturbance.

Little is known at present about the mode of action of molybdenum. It is significant that apparently only ruminants are affected. In the early part of this investigation it had been hoped to use a small animal, such as a rabbit or guineapig, for various tests. In trials, however, 160 mg. molybdenum, as sodium molybdate, were given to rabbits without causing any change in the consistency of the faeces. This dose on an equivalent bodyweight was about 8 times that which caused severe scouring in dairy cows.

Molybdenum appears to be eliminated quickly and completely by sheep, and experiments in which teart grass and molybdenum salts were administered showed that 99 per cent. of the molybdenum was excreted within a few days. The absorption from the alimentary tract was considerable, since up to 50 per cent. of the molybdenum appeared in the urine.

Since an appreciable part of the molybdenum evidently enters the blood stream, the possibility was considered of its interfering with the function of the blood copper. To test this 6 cows in milk were given daily doses of molybdenum so that they were decidedly loose but not scouring. Blood samples were taken at weekly intervals for 4 weeks, but the analyses showed that the molybdenum had had no effect on the blood content of copper, the average initial and final figures being, respectively, 0·126 and 0·128 mg. per 100 ml. blood.

All our observations point to the rumen as the centre of at least the initial disturbance. We had hoped to complete an experiment in time for this meeting in which the rumen was to be avoided by injecting molybdenum subcutaneously into dairy cows, the quantity of molybdenum being similar to that which would cause scouring when given orally. If no scouring occurred, this should prove that molybdenum does not exert its influence directly through the blood stream and should provide evidence that the rumen is the seat of the trouble. Unfortunately some difficulty has been experienced in getting the injected molybdenum to disperse, and the experiment has to be repeated.

There is evidence that molybdenum has some influence on the behaviour of certain soil bacteria, probably connected with nitrogen metabolism, and it seems possible that some of the numerous organisms present in the rumen might also be affected. The production of toxic substances by these organisms might be an explanation of the physiological

disturbance in cattle, but we have not been able to follow this line of investigation.

If we knew how molybdenum acts we might be nearer to an explanation of the curative effect of copper sulphate.

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Discussion

Dr. S. J. Watson (Jealott's Hill Research Station, Bracknell, Berks.), opener: There is little to be added to the details which Mr. Ferguson has given in his paper. An interesting result of the valuable effect demonstrated for copper sulphate has been the issue to farmers on teart farms in Somerset of a cattle cake containing this salt. As a result, large areas of grassland which could not otherwise have been grazed were used for milk production. An improvement in the condition of cattle receiving the supplement is evident from the reports of a number of users of the cake, who found that the scouring had been entirely checked.

Sir J. Barcroft (Physiological Laboratory, Cambridge): Is the ratio of urinary to faecal excretion of molybdenum the same in animals which receive copper as in those which are not treated?

Mr. C. N. Bromehead (H.M. Geological Survey, Exhibition Road, London, S.W.7): Could Mr. Ferguson or his colleagues give any particulars of the distribution of teart on the Lower Lias outside the type area in Somerset or in a region, Carrock Fell, in Cumberland, where molybdenite is present?

Dr. A. H. Lewis (Jealott's Hill Research Station, Bracknell, Berks.): Teart land occurs in this country on outcrops of the Lower Lias, in Somerset, Gloucester, Warwick and apparently Lincoln, but not in Glamorgan. This difference is apparently due to differences in lithological character of the Lower Lias. Molybdenum seems to be concentrated in the clay component of the Lower Lias; the limestone contains little. In the teart areas the Lower Lias is predominantly argillaceous whereas, in Glamorgan, limestone is predominant and the upper argillaceous layer is missing. Teartness has not been reported from other parts of the country.

Even in Somerset all the soils derived from the Lower Lias are not teart. Where the soils are acid or slightly acid in reaction, the molybdenum in the soil is only absorbed with difficulty by pasture plants; on neutral or alkaline soils absorbtion of molybdenum by pasture plants is rapid. The addition of acidic substances to teart soils reduces uptake of molybdenum by the herbage and so reduces its teartness.

All pasture species do not absorb molybdenum with equal ease; leguminous plants, such as clovers, absorb considerably more than grasses. Hence, the more clover there is in a pasture, the greater is its effect in causing scouring in cattle.

Dr. K. P. Oakley (H.M. Geological Survey, Exhibition Road, London, S.W.7): A. P. Vinogradov's compilation dealing with the chemical composition of marine organisms, published by the Biogeochemical Laboratory

of the Academy of Sciences of the U.S.S.R. (Vinogradov, 1935) makes it evident that molybdenum is not concentrated from sea water by any known invertebrate animal, but that some algae, notably *Laminaria*, contain conspicuous traces of the element. It is, therefore, of interest in connexion with the relatively high concentration of molybdenum in some beds of the Lower Lias that the fauna of minute gastropods in this formation is similar in facies to that associated with *Laminaria* in present day seas. This suggests that parts of the sea floor in Lower Liassic times were covered by beds of *Laminaria* which might have concentrated molybdenum from the sea water and led to its fixation in the bottom muds.

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Mr. W. S. Ferguson:

In reply to Sir J. Barcroft: The effect of copper on the ratio of urinary and faecal excretion of molybdenum has not been studied.

The Absorption and Excretion of Trace Elements

Dr. E. M. Widdowson and Dr. R. A. McCance (Department of Medicine, Cambridge)

There is nothing special about the absorption and excretion of trace elements. They behave, on the whole, as one would expect from a knowledge of their chemical properties, and of the way in which the more abundant physiological elements with similar chemical properties are absorbed and excreted. It would, therefore, be foolish to discuss the behaviour of trace elements without referring to the more abundant ones such as calcium, phosphorus and iron.

The absorption and excretion of elements is to some extent a matter of real physiology, as is instanced by the way in which vitamin D influences the absorption of calcium, and the hormone of the suprarenal cortex, the excretion of sodium, chloride and potassium. To a large extent, however, the rules that govern absorption and excretion are those of pure chemistry, and elements with similar chemical properties tend to behave in a similar way, even though their physiological functions are widely different. Chlorides and iodides illustrate this point.

To be absorbed an element must be in solution, and for this reason it is, generally speaking, the most soluble ions that are most readily absorbed. Physiological differences, however, come into play, for the intestine seems to find it much less easy to absorb divalent than monovalent ions, even if they are equally soluble. Thus, magnesium chloride is more soluble than sodium chloride, but it is certainly not as well absorbed. Radicals which precipitate an element in the intestine will impede its absorption. There are many examples of this. Calcium is not well absorbed, partly because it is divalent, but also because it forms a relatively insoluble phosphate, and an insoluble phytate and oxalate. Most other divalent elements form insoluble phytates; iron, zinc, magnesium and manganese are examples, and this property sometimes limits their absorption. A sure way of preventing the absorption of

phosphorus is to add beryllium to the diet, because beryllium phosphate is completely insoluble. Conversely, beryllium taken by mouth, could never be absorbed in more than traces as long as the diet contained phosphates.

On the other hand, anything which improves the solubility of an element in the digestive juices will, *ipso facto*, tend to increase its absorption. We have found that the absorption of calcium and magnesium is influenced by the amount of protein in the diet, because calcium and magnesium phosphates and carbonates, themselves insoluble at the *p*H of the intestine, tend to form soluble co-ordination compounds with the amino-acids. It will quite probably be found that co-ordination compounds of this type play a part in the absorption of metals other than calcium and magnesium, though iron does not seem to behave in this way.

All elements which are freely absorbed appear to be freely excreted by the kidney, and it is this organ which regulates the amount retained within the body. This is true, whether the element is present in traces like lithium and rubidium, or in large amounts like sodium and potassium. The kidney will excrete any element which is presented to it in true inorganic solution. It will, for example, excrete cobalt, nickel and tin, or soluble gold and bismuth salts when these are injected into the body, but it does not appear to excrete insoluble gold or bismuth compounds if these are injected, nor does the healthy kidney in any circumstances excrete iron, zinc or manganese.

A slight digression must be made about the way in which the intestine functions. Food is absorbed from the intestine, part of whose function it is to digest the food and, in order that it may do this, enormous quantities of fluid are poured into it every day. These solutions are all isotonic with the plasma, and contain sodium, chloride and small amounts of potassium, calcium and magnesium and other elements. All these solutions are highly specialized, and some of them, such as the bile, are vehicles of excretion as well as of digestion. It is, therefore, difficult, even with the aid of radio-active isotopes, to measure the real absorption of any element which enters the intestine with the digestive juices, and equally difficult to measure the amount of any element excreted into the intestine as long as that element is also being absorbed. All that can be done is to measure the amount in the food and the amount in the faeces, and the difference between these two quantities represents the net gain or loss. If the amount in the food is greater than the amount in the faeces, the element is being taken up from the gut. If the faeces contain more than the food, more of the element is finding its way out of the body into the intestine than is being absorbed. This is just as true of sodium and potassium as it is of zinc and lead. Whether, therefore, an element appears at a given time to be excreted or absorbed by the intestine depends on the amount in the food, the ease with which it is absorbed, and the quantity of it which is excreted into the gut. If conditions were suitably arranged, it could probably be demonstrated quite easily that any element was being excreted into the intestine, or was being absorbed by it.

The laws of absorption and excretion are largely chemical laws, so that reference to the periodic table will help to give a general idea of the way in which trace elements are absorbed and excreted. First of all,

there are those elements which are readily absorbed and as readily excreted in the urine. These are the alkali metals in Group 1 and the halogens in Group 7. They are all monovalent elements. Both groups contain trace elements and common elements. All are absorbed to the extent of well over 90 per cent. The soluble milligram or so of lithium in the daily food is treated exactly like the 5000 milligrams of sodium.

Next come those divalent metals which are absorbed, but with considerably more difficulty than the monovalent alkali metals or the halogens. About 50 per cent. of the amount in the food is taken from the intestine, but the proportion for the different elements varies from 20 to 70 per cent. Most of the fraction which leaves the intestine and enters the body is excreted by the kidney, but a small fixed quantity, which does not vary with the amount absorbed, finds its way back into the intestine with the digestive juices. If these metals are given intravenously they are excreted in the urine, though, of course, traces find their way into the intestine in the digestive juices. This group includes copper, magnesium, calcium, strontium, tin, cobalt and nickel. Barium would probably also fall into this group, but barium salts are highly toxic, and little is known about their absorption and excretion. There are only traces in the food, but cattle must obtain barium from somewhere, for 1·5 per cent. of the dry matter of the choroids of their eyes consists of barium. Barium provides an excellent example of an insoluble compound which is not absorbed. Tons of this toxic metal must be administered every year to patients in the X-ray departments of hospitals. Their lives depend on the insolubility of barium sulphate.

The next group of elements to be considered includes those which are absorbed with very great difficulty and, even when they are injected into the blood stream in appreciable amounts, are not excreted by the kidney in more than the merest traces. In the body these elements are probably attached to the proteins in the serum, and do not, therefore, pass through the glomerular membranes of the kidney. Zinc, manganese and iron come into this group. Cadmium has no physiological function for any species except the common scallop, which seems to make use of it, and there appears to have been no recent study of its absorption and excretion. It is, however, closely related chemically to zinc, and it seems likely that it would behave like zinc. It also precipitates serum proteins *in vitro* and, if it were injected intravenously, the kidney would probably not excrete it.

Since these elements in the serum are probably attached to the proteins, it seemed reasonable to suggest that patients with albuminuria might excrete them with the protein. Accordingly, we determined the amount of zinc in some 24 hour specimens of urine from patients with albuminuria, and found at least ten times as much as in 24 hour specimens from normal people.

The intestine seems to play an important part in the excretion of zinc, lead and manganese. We believe, however, that it does not regulate the excretion of iron and that, once iron gets into the body, there it tends to remain.

Injected radio-active zinc has been shown to be excreted mainly by the pancreas in the pancreatic juice, and manganese by the liver in the bile. As stated earlier, an element must be in solution to be absorbed

and, since the digestive tract always contains chlorides, it would not be expected that an element which forms an insoluble chloride would be readily absorbed. So far as is known, mercury is not a physiological metal, but it behaves in the expected way. Mercurous salts are not absorbed because mercurous chloride is insoluble. Mercuric chloride is soluble, and mercuric salts, when taken by mouth, are absorbed and slowly excreted by the kidney. Bismuth forms a very insoluble oxychloride and this salt was used at one time as the opaque material for X-ray diagnostic examination of the gastro-intestinal tract. Although silver salts taken by mouth are not absorbed in significant amounts, silver does sometimes gain entrance to the body through the skin and mucous membranes. An unfortunate lady who mistook her doctor's orders, continued to wash out her nose every day for 10 years with a preparation of silver. The silver gradually penetrated the mucous membranes, and as it was carried over the body it was deposited in the skin as the insoluble chloride. We did a metabolic experiment on this lady and found that she was not excreting any of the silver with which she was so saturated.

Discussion

Dr. N. L. Kent (Ministry of Food, Cereals Research Station, Old London Road, St. Albans), opener: The absorption of trace elements is governed not only by chemical but also by physiological laws, hence, for example, differences in absorption of manganese by different human subjects and differences in absorption of the same inorganic salts of manganese by plants and by man. More information is required about the trace element content of foodstuffs and of blood in normal conditions and after ingestion of trace elements. The state of these elements in blood should be examined to determine whether they are present as inorganic ions or as metallo-proteins. The effect of certain elements on the absorption of other elements is another subject for enquiry. The toxicity of poisonous elements depends on the foodstuff in which they are present; thus lead in bread is probably in the form of phytate and therefore harmless.

Dr. T. Mann (Molteno Institute, University of Cambridge): The investigation of radio-active isotopes promises to be very helpful in the study of absorption of inorganic elements. Thus radio-active iron could be demonstrated combined as haemoglobin inside the blood corpuscles a few hours after administration to an experimental animal.

Dr. R. A. McCance gave the following reply:

Radio-active elements are proving increasingly useful but the results require careful interpretation. Thus, if radio-active sodium is found in the faeces after intravenous administration, it does not mean that the intestine plays any part in ridding the body of the excess of sodium caused by the injection. This is done by the kidney. The radio-active sodium reaches the intestine by displacing ordinary sodium from the gastro-intestinal secretions, and a little remains there with the sodium always found in the faeces.

Concluding Remarks by the Chairman

Dr. C. R. Harington (National Institute for Medical Research, Hampstead, London, N.W.3): The only excuse which I can find for my presence in the chair is that I have myself been interested for many years in an element which might be considered a trace element in the sense of the day's discussion. Iodine is an element widely distributed, indeed almost ubiquitous, in nature; it never occurs, however, in high concentration, and it is known to be essential to normal animal life. It, therefore, seems to satisfy the main criteria which have been laid down in the attempt to define a trace element in the biological sense.

In the course of my study of the historical development of the subject I have become continually more impressed with the necessity of the completion, from all points of view, of the evidence concerning the function of an element supposedly of biological importance, before a full and satisfying picture can be presented. It was not long after the discovery of iodine before an association between deficiency of this element and the occurrence of endemic goitre was suspected, and evidence, which in retrospect seems fully satisfying, was rapidly accumulated in favour of such an association. Nevertheless, owing perhaps to the strangeness of the idea of deficiency as a cause of disease, this evidence failed to carry conviction. The present general acceptance of the simpler relationships between iodine and thyroid function was not indeed established until comparatively recent times, and is dependent on three additional pieces of evidence, the proof of the occurrence of iodine in the thyroid gland, the observations of Marine (*cf.* Marine, 1922) on the relationship of the iodine content of the gland to its histological structure and, finally, the demonstration that iodine is a constituent of the active principle. Thus, the position of iodine as a trace element in the biological sense was not established by the demonstration even of an apparently causal relationship between its presence or absence and thyroid function; it was necessary also that the evidence should be completed by the detailed explanation of its physiological function and mode of action.

I see in the story of the development of knowledge of iodine in its biological relationships certain considerations which are relevant to the day's discussion. The trace elements of which the various contributors have spoken seem to fall into two main categories: those which are present in soil or water, and cause toxic effects when they occur in abnormally high concentration, and those which occur as minor but apparently essential constituents of the animal body.

About the first group there seems little that can usefully be said at the present time. If the second group is considered, however, it appears that, whilst in many cases no specific function can be assigned to the trace element, there are others in which the biological role of the element can be clearly defined. Examples of this are copper and zinc, both of which are now known to be associated with enzyme systems essential to the life of animals or of plants on which animals depend for food. It appears to me that such trace elements are entitled to the same full recognition, in respect of their biological importance, that is now generally accorded to iodine; it is essential to realize, however, that final conviction in the matter, at least as far as the outside scientific observer is concerned,

rests ultimately on the demonstration of the actual biological role of the element in question.

I urge, therefore, that, in further work on trace elements, more attention should be given to the study of their mode of action in the animal or plant. These considerations apply chiefly to the essential trace elements; it is, however, also true that no satisfactory picture of the toxic trace elements can be presented until the mechanism by which they exercise their toxic effect can be precisely defined. Here also, therefore, there is the need for detailed study of biological action rather than for the mere accumulation of evidence of causal association between environmental presence of excess of a certain element and the occurrence of a corresponding disease of animals or plants.

REFERENCE

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