CLXXIII. A SPECTROGRAPHIC ANALYSIS OF HUMAN TISSUES.

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THIS paper describes the results of a spectroscopic examination of 195 specimens of different human tissues, both healthy and diseased. The object of the investigation was to determine whether any chemical elements were constantly present in the tissues in addition to those already recognised as being universal constituents of protoplasm (C, H, O, N, P, S, Na, K, Mg, Ca, Cl, Fe, Cu) and to discover what variations might occur both between different organs and between healthy and diseased tissues.

PREPARATION OF THE MATERIAL.

A general description of the method has been published by one of us [Ramage, 1929]. Small pieces of the organ were removed and washed in distilled water to free them from blood as far as possible. They were then placed in a steam-oven and dried until of constant weight. In cases where the presence of fat made this difficult, the melted fat was removed by drying them on the ashless filter-paper used for dealing with fluid specimens. The resulting material was then ground to powder in an agate mortar, and a small amount of this powder was used for obtaining the spectrum. Except where it is otherwise stated, the amount of each specimen that was used was 0·05 g. This was rolled in a small piece of ashless filter-paper—weighing about 0·12 g.—cut from Munktell's Swedish paper No. 00 diameter 12·5 cm., and the whole was then burnt in an oxy-coal-gas flame before the slit of a quartz spectrograph, a quartz lens being used to focus the image of the flame on the slit. The spectrum of the flame itself has no metallic lines, but shows a number of lines in the ultra-violet due to water-vapour. The spectrum of the filter-paper shows very weak lines due to sodium, potassium, and calcium, but apart from these is devoid of metallic lines. The spectrograph employed was a Size C Hilger quartz spectrograph, and the photographic plates have been Ilford panchromatic coated on thin glass. Plates 5″ by 4″ suitably placed in the holder cover the region from the red to beyond λ2800, which is that required when no arc is used. In the case of fluids such as blood, 0·1 cc. was transferred to a piece of the filter-paper which had previously been rolled from the curved edge, dried in the steam-oven, and burnt in front of the spectrograph in the same way as the solid specimens.

Standard solutions were prepared having the following percentage composition: (1) Na, 0·15; K, 0·20; Ca, 0·02; Li, 0·0004; Rb, 0·004; Mg and Fe, 0·05; Cu and Co, 0·005; Mn and Ni, 0·002; Cd, 0·10; P, 0·50; and (2) Sr, 0·002; Ag, 0·0025; and Pb, 0·05. The brilliance of the lines obtained on burning papers charged with the standard solutions naturally varies with the amount of solution used, and since it is possible to obtain the photographs of 16 spectra on the one photographic plate, four of these were devoted to the standard solutions, each in amounts of 0·02, 0·05, 0·1 and 0·2 cc. In this way, by matching the intensity of the lines of an element in a specimen with that in the different strengths of standard solution, the method can be put to a quantitative as well as a qualitative use.

Though figures are given throughout this paper, it is not desired to lay stress at present on the quantitative nature of the investigation, the original purpose of which was a qualitative survey of the metallic constituents of the various tissues. Only the four strengths of standard solution already mentioned were employed, and in consequence, where the brightness of the lines of a particular element is less than that shown by the element in the weakest strength of the standard solution, a "trace" of the element in question has usually been said to be present. In using the method further for quantitative purposes, either a more extensive range of variation of the strengths of standard solution could be employed, or the standard weight of specimen burnt (0·05 g. here) could be altered, so that the lines could be accurately matched.

Except in the case of blood, all the percentages given are in terms of the dry weight of the tissue. Where the term "trace" is used, the element in question is present in a percentage of less than the following, which are the percentages represented (when 0·05 g. of specimen is used) by lines matching those formed by the burning of 0·02 cc. of standard solution.

	%		%
Sodium	0·06	Manganese ...	0·0008
Potassium ...	0·08	Iron	0·02
Lithium	0·00016	Silver	0·001
Calcium	0·008	Strontium ...	0·0008
Rubidium ...	0·0016	Lead	0·02
Magnesium ...	0·02	Phosphorus ...	0·20
Copper	0·002		

By this method the following elements in addition to those mentioned above may be readily detected when present in 0·05 g. of a specimen: caesium, barium, cadmium, gallium, indium, thallium, bismuth, chromium, cobalt, nickel, ruthenium, rhodium, palladium and iridium. Other elements have been described as occurring in plant or animal tissues which are not included in this list, of which the more important are zinc, aluminium, tin and titanium. These elements can be detected when an arc spectrum is employed, and in a recent paper by Zbinden [1930] were all described as occurring in human tissues. Zinc has been described by Bodansky [1921] as a normal constituent of the brain. The reason why these elements cannot be detected directly by the method employed here has already been given by Fox and Ramage [1931], *viz.* "that the thermal or electrical conditions of the flame are not sufficiently intense either (*a*) to reduce the oxides in adequate quantities or (*b*) when small quantities only are present, to excite the vibrations producing the lines with enough energy to photograph them, placed, as most of them are, in the further portion of the ultra-violet region of the spectrum, *i.e.* beyond wave-length 3100." The arc spectrum employed by Zbinden, making use of carbon electrodes, while more sensitive, introduces a risk of error due to impurities in the carbons *etc.*, which is not present in our method.

In view of the fact that the presence and distribution of sodium, calcium, potassium and magnesium through the body is already well known, these metals are not referred to except where they show some striking anomaly.

The cases from which tissues were taken for examination may be summarised as follows:

S. Male, age 77. Death from cerebral haemorrhage, due to arteriosclerosis. The heart was enlarged and adherent to the pericardium. The pericardial adhesions were calcified in places, and the mitral valve also showed calcified nodules.

F.M. Female, age 69. Death from cholangitis, following chronic inflammation of the gall-bladder.

K.T. Female, age 2. Death from acute broncho-pneumonia.

A.B. Male, age 35. Death from pneumonia following operation for a perforated duodenal ulcer.

G.E. Male, age 53. Squamous-celled carcinoma of the oesophagus.

N.C. Female, age 27. Death from acute tubercular broncho-pneumonia. Both lungs showed widespread tubercular deposits, without obvious caseation. The thyroid gland contained a small adenoma.

M.R. Female, age 7. Acute broncho-pneumonia.

M.L. Male, age unknown. Advanced chronic interstitial nephritis.

E.C. Female, age 5. Death from diphtheritic toxaemia.

G.C. Male, age 9 months. Acute broncho-pneumonia.

R.M. Male, age 61. Death from uraemia.

H. Male, age 25. Miliary tuberculosis.

Foetus D, 24 weeks development. This was a normal foetus, which was removed at operation by Mr Maslen Jones, owing to heart disease of the mother.

Foetus D (*a*), 24 weeks development. Abortion of unknown origin.

Foetus F, female, full-term development. Stillborn.

Foetus (10), foetus of 10 weeks' development. Abortion.

L. Female, age 20. Chronic lymphatic leukaemia.

J. Male, age 72. Advanced atrophic cirrhosis of the liver, which was probably alcoholic in origin.

E.B. Male, age 49. Death from tuberculosis with advanced silicosis of the lung.

P. Male, age unknown. Advanced silicosis of the lung.

L (2). Female, age unknown. Myeloid leukaemia, death occurring while 26 weeks pregnant.

Foetus L (2). The foetus of the above case.

B.P. Age 9 months. Death from miliary tuberculosis.

G.P. Owing to the difficulty of freeing the human tissues removed at *post-mortem* from all their contained blood, this was done in the case of a guinea-pig. The animal was killed by bleeding,

and immediately after death a cannula was inserted into the aorta and the animal transfused with distilled water. The organs, after being dissected out, were minced and again washed in glass-distilled water before being placed in the drying oven.

Description of the various tissues.

Table I. *Liver.*

Patient	Copper	Manganese	Rubidium	Silver
S.	Trace	0·0004	0·002	Trace
F.M.	0·0015	0·0004	0·002	Trace
A.B.	0·002	0·0005	0·003	Trace
G.E.	0·003	0·0008	0·004	Trace
N.C.	0·0015	Trace	0·0016	—
Foetus D	0·035	0·001	0·004	Merest trace
Foetus (10)	0·015	Trace	None	Merest trace
J.	0·02	0·0008	0·0016	—
E.C.	0·002	0·0008	0·0024	Trace
Foetus F	0·025	0·001	0·0016	Trace
Foetus D (*a*)	0·03	0·0004	0·002	—
G.C.	0·002	Trace	0·002	—
R.M.	0·02 +	0·0008	0·004	—
Foetus L (2)	0·02 +	Trace	0·0016	—
L (2)	0·002	0·0008	0·0016	Trace
B.P.	0·02	Trace	0·0024	—
G.P.	0·0075	0·002	0·004	—

Liver. The iron spectrum was rich in all the specimens examined, including that of the transfused guinea-pig. The lines of Na, K, Ca and Mg reveal no unusual features, except that the liver of the 10-weeks foetus was very high in Ca and very low in Mg and K. Copper was present throughout, and, in general, the lines indicate a higher concentration in this organ than in any other region of the body; *e.g.* in the transfused guinea-pig, 0·0075 % in the liver, as against slightly less than 0·002 % for the other organs of this animal that were examined. The most prominent feature of the distribution of copper in the liver as shown by Table I is the high Cu content of the foetal liver, the lowest amount, found in the 10-weeks specimen (0·015 %), being some three times as much as that of the average figure (0·0045 %) for the adult livers. This particular specimen was in an advanced state of autolysis when dissected out, and it was only possible to obtain 34 mg. of dry tissue for burning. The other four foetal specimens have an average of 0·0275 %. The difference is strikingly shown by the case of L (2) where the maternal liver had 0·002 % Cu and the foetal liver more than 0·02 % Cu. That there is considerable variability in the rate of dispersion of copper from the liver after birth is shown by the difference between the Cu contents of the livers of two 9 months old babies (G.C. 0·002 % and B.P. 0·02 %). The high Cu content of the foetal liver is probably connected with its blood-forming functions in this stage of life, since it has been shown by Waddell *et al.* [1928, 1929] that the presence of this element is necessary for the formation of haemoglobin.

Specimens J. and R.M. are of interest, since these were the only adult livers to give copper lines of an intensity comparable with those found in the foetus. In the case of J. the liver was examined in the first place with the idea that it might contain an abnormal amount of manganese, in view of the facility with which cirrhosis of the liver can be produced in experimental animals by this metal. The manganese line was, however, weak, but the copper lines were very strong (0·02 %). Whether the presence of this large amount of copper bears any relation to the development of the disease is uncertain. Schonheimer and Oshima [1929] state that cirrhosis of the liver may occur with a normal amount of copper. The case of R.M. (uraemia) is of importance in the reverse connection, since the liver contained slightly more than 0·02 % of copper, while the organ itself, though large, was normal in appearance except for some fatty degeneration, and showed no sign of cirrhosis. Manganese was found in every liver, and in five instances the amount present was less than 0·0008 %. The organs of the baby G.C. who died at the age of 9 months from broncho-pneumonia, had, in general, a low content of copper and manganese, which raises the interesting speculation as to whether a deficiency of these metals plays any part in lowering resistance towards infection. The transfused guinea-pig gave the strongest spectrum (0·002 %) while among the human specimens foetus D and foetus F had the greatest amount (0·001 %). As with copper, the liver apparently has a greater need for manganese during foetal life than after birth. The amount of manganese was low in both foetus (10) and foetus D (*a*), but both of these died from unknown causes, while foetuses F and D were healthy.

Rubidium was present in every liver except that of the 10-weeks foetus. The lines were generally strong, though there were considerable individual variations, the greatest amount in the adults (0·004 %) being found in G.E. (carcinoma of the oesophagus), R.M. (uraemia) and in the liver of foetus D.

Traces of silver occurred in 9 of the 17 specimens of liver. A trace of lead was found in the case of G.E. and the liver of the 10-weeks foetus was unique in containing a trace of strontium. It is worthy of note that 3 of the 5 foetal livers contained traces of silver.

Gall-bladder and bile. Four specimens were examined. (Gall-bladders G.E. and foetus F, bile from foetus F, and both gall-bladder and bile were examined together from foetus D. The bile was dried before examination.)

The gall-bladder of G.E. had strong Ca lines, presumably the result of age. The Cu lines were strong in both gall-bladder and bile of foetus F, the gall-bladder containing 0·0075 % and the bile 0·02 %.

The merest trace of manganese was found in the gall-bladder of G.E., while in foetus F the bladder gave a strong line (0·0015 %) and in the dried bile the metal was present in even larger amount (0·008 %). Faint lines of rubidium were found in all the specimens. The bile of foetus F showed traces of silver, this metal being also found in the liver.

Pancreas. From Table II it will be seen that copper was present in every specimen examined, but that in only one case, that of the full-term foetus, did it rise to 0·002 % of the dry weight. Manganese was found in all cases, and also rubidium, the lines of which are visible in every spectrum of the pancreas, but nowhere represent a value of more than 0·0024 %. The rubidium lines are weakest in the foetal specimens. Traces of silver were found in three cases. The strength of the manganese lines is the most striking feature of the examination of this organ.

Table II. *Pancreas.*

Patient	Copper	Manganese	Rubidium	Silver
S.	Trace	0·0008	0·0016	Trace
F.M.	Trace	Trace	Trace	Trace
A.B.	Trace	0·001	0·0016	Trace
G.E.	0·002 (almost)	0·0008	0·0024	—
N.C.	Trace	0·0008 (almost)	0·0016	—
Foetus D	0·002	0·0008 (almost)	0·0016	—
Foetus F	0·002	0·001	0·002	—
Foetus D (*a*)	Trace	Trace	Trace	—
G.C.	Trace	0·0008	0·0016	—
R.M.	Trace	0·0008	0·0024	—

Table III. *Spleen.*

Patient	Copper	Manganese	Rubidium	Silver	Other elements
S.	Trace	—	0·0016	—	—
L.	Trace	—	0·0016	—	—
F.M.	Trace	—	0·0016	—	—
K.T.	0·002	0·0008	0·001	—	Trace of lithium
A.B.	Trace	—	0·0024	—	—
G.E.	Trace	—	0·004	Trace	—
N.C.	Trace	Trace	0·0024	—	—
E.C.	Trace	—	0·004	—	—
Foetus D	Trace	Trace	0·005	—	—
Foetus F	0·002	—	0·004	—	—
Foetus D (*a*)	Trace	—	0·004	—	—
G.P.	Trace	Trace	0·006	—	—

Spleen. As was expected, the iron lines were very strong in the spectra of this organ. The magnesium lines showed nothing remarkable. Copper was present in every specimen, reaching a concentration of 0·002 % in K.T. (a girl age 2) and in the full-term foetus. Manganese was only found in 5 out of the 12 spleens examined. In the highest (K.T.) it only reached 0·0008 %, while in all the others it was present in the merest traces. Rubidium occurred in all specimens. In the transfused guinea-pig it reached the high value of 0·006 %, and it was nearly as high in the foetuses. In the blood rubidium was found in the red cells, and the destruction of these by the spleen may account for the richness of the spleen in this element. Silver occurred in one case only, and a trace of lithium was found in the spleen of K.T.

Kidney. In the guinea-pig and in foetus D the potassium lines were of the same intensity as in the organs already described, but in all the other specimens they were distinctly weaker. The magnesium line was strong in foetus D (0·2 %), but in all the other specimens represented 0·1 %. Traces of copper were present in every specimen, reaching the concentration of the weakest standard solution in four cases, two of which were foetal specimens. Manganese was absent from the kidneys of the two foetuses which died

Table IV. *Kidney.*

Patient	Copper	Manganese	Rubidium	Silver	Other elements
S.	Trace	Trace	0·0024	—	—
L.	0·002	Trace	Trace (faint)	—	—
F.M.	Trace	Trace	0·0024	—	—
K.T.	Trace	Trace	0·0024	—	—
A.B.	Trace	0·0008	0·003	—	—
G.E.	Trace	Trace	0·004	Trace	Lead (trace)
E.C.	Trace	Trace	0·003	Trace	—
Foetus D	0·002	Trace (faint)	0·005	—	—
Foetus (10)	0·002	—	—	—	Strontium (trace)
Foetus F	Trace	Trace	0·003	Trace	—
Foetus D (*a*)	Trace	—	0·0024	—	—
G.C.	Trace	0·0008	0·0024	—	—
G.P.	0·002	0·0008	0·004	—	—

in utero, but was present in small amount in all the other kidneys. Rubidium was absent from the kidney of the 10-weeks foetus and almost so from L. (myeloid leukaemia), but was present in significant amount in the other specimens. Traces of silver were found in three of the kidneys and lead in one. The kidney of the 10-weeks foetus was remarkable for containing a trace of strontium.

Table V. *Suprarenals.*

Patient	Copper	Manganese	Rubidium	Silver
S.	0·002	0·002	Trace	Trace
F.M.	Trace	Trace (faint)	Trace	Trace
K.T.	Trace	Trace (faint)	Trace	Trace
E.C.	Trace	Trace	0·003	Trace
Foetus D	Trace	Trace	0·006	—
Foetus F	Trace	Trace	0·0016	—
Foetus D (*a*)	0·002	Trace	0·004	—
Foetus L (2)	Trace	Trace	0·0016	—
G.P.	0·002	0·001	0·004	—

Suprarenals. Copper occurred throughout in small amount. Manganese was found in every case, but the variations were extraordinary (from a trace to 0·002 %). Rubidium occurred in small quantities in the post-natal specimens, while in the foetal ones measurable quantities were present. Silver was absent from the guinea-pig and foetal suprarenals, but occurred in traces in the others.

Urinary bladder. This organ was examined in two cases (A.B., G.E.) A trace of copper was present in each. Manganese occurred in A.B. as an obvious trace, while in G.E. the manganese line was only just visible. Rubidium was found in both specimens.

Intestinal canal. The stomach of F.M. and the duodenum of A.B. were examined. Traces of copper, manganese, and rubidium were found in both. A gastric ulcer removed by partial gastrectomy, compared with the adjacent normal stomach, showed a difference only in rubidium (normal 0·006 %, ulcer 0·0024 %), and in copper, in which element the ulcer was slightly richer. The Mn line in both cases was below the brightness of 0·0008 %. It is hoped to pursue this line of investigation in other cases.

Prostate. (M.L.) This organ contained traces of copper and silver, with 0·002 % of rubidium, but otherwise showed no unusual features.

Testis and epididymis. (M.L.) Both these organs contained traces of copper and the faintest traces of manganese. Rubidium was found in each (0·0016 %).

Uterus. (N.C., F.M.) The two specimens examined revealed no unusual features. Both contained traces of copper. Manganese was present as a trace in the uterus of F.M. but not of N.C. Rubidium was present in N.C. in a concentration of 0·0024 %, while in F.M. only a trace was found.

Placenta. The healthy 24-weeks placenta belonging to foetus D and that of foetus L (2) (myeloid leukaemia in the mother) were examined. Traces of copper and rubidium were present, but there was no manganese.

Ovary. (F.M., N.C., M.R.) The K lines were weak in F.M. Copper was present in small amount in all three, but the lines were distinctly stronger in the two patients who were below the age of the menopause. Manganese was absent, except for the merest trace in one of the ovaries of M.R. Rubidium gave a faint line (0·0016 %) in F.M. and slightly stronger lines (0·0024 %) in the other two. While there is obviously insufficient material for a generalisation, it is to be noted that the ovaries whose functional activity had not ceased were distinctly richer in potassium, copper, and rubidium, and a re-examination of the spectra shows that the same is also true of magnesium. There was, however, no difference between the spectra of the ovaries of the girl of 7 and of the adult of 27. The nature of any such changes in the mineral content of the ovaries which may set in after the menopause is under investigation.

Thyroid. (S, M.R., M.L., N.C. In the last case both the normal gland and a small adenoma were examined.) Copper was found in every case, but only in M.R. did it rise to a value of 0·002 %. Manganese was present in minute quantities in S. and in both specimens from N.C. but was absent from the other two. The rubidium lines were remarkably constant in all five specimens (0·002 %). Traces of silver were found in all the specimens. The normal gland was slightly richer in copper and the adenoma in manganese, but the differences were very small, and apart from these the spectra were identical. The thyroid of M.R. showed phosphorus, the lines of which were not visible in the other spectra.

Thymus. (Foetus D and foetus F.) The Mg line was stronger in the full-term foetus and the Cu lines were very much stronger (0·002 % as against a trace). The younger foetus, on the other hand, contained a trace of manganese, which was not present in the thymus of the elder. The Rb lines were strong in both (0·006 % in foetus D and 0·004 % in foetus F).

Tonsil. Four tonsils removed from children were examined. The spectra revealed practically no individual differences, but their general nature is interesting. Traces of copper were present in all but no manganese was found. The Rb lines were strong (0·003 %) and present in all. Every tonsil contained traces of silver.

Skin. The adult skin has not been examined, but the skin of foetus D contained 0·0024 % of rubidium, with traces of copper and manganese.

Heart. In all cases, pieces of heart muscle from the left ventricle were examined.

Table VI. *Heart.*

Patient	Copper	Manganese	Rubidium	Silver
S.	0·002	Trace	0·004	—
F.M.	0·002	Trace	0·004	—
K.T.	Trace	—	0·003	—
A.B.	Trace	—	0·004	—
G.E.	Trace	Trace	0·003	Trace
N.C.	Trace	Trace	0·003	Trace
E.C.	0·002	—	0·003	—
Foetus D	Trace	—	0·006	—
Foetus F	Trace	Trace	0·004	—
G.C.	Trace	—	0·002	—
R.M.	Trace	Trace	0·003	—
L (2)	0·002	—	0·0016	—
G.P.	0·002	Trace	0·006	—

Copper occurred throughout, but in only four human specimens was it more than a trace. Manganese was found in traces in six cases. Rubidium, on the other hand, gave lines that were remarkably constant in strength, corresponding to an average content of 0·0037 %. Silver was found in two of the specimens. No significant differences could be detected in the spectrum of E.C., death in this case being due to toxic myocarditis following diphtheria.

Aorta. (S., F.M., N.C.) As compared with N.C. (age 27) the Ca lines of S. (age 77) and F.M. (age 69) were markedly stronger, and, as is found in practically all the cases where the Ca lines are strong, the Mg line was much weaker. These changes are presumably the result of age. The merest traces of copper and rubidium were found in all three specimens. Manganese was only found in the aorta of S., and, as has been already mentioned, it gave a remarkably strong line (0·002 %). This aorta contained traces of silver and lead. Apart from any significance which may attach to the peculiar richness of this aorta in manganese, the presence of silver and lead in addition was presumably connected with the advanced degree of atheromatous change with calcification that was found.

Calcareous nodules from the case of S. were examined. These were found in the pericardium and on the mitral valve. The nodule on the valve was very small (0·168 g. dry weight) and it is possible that its general poorness in metals may be due to its small size making the traces present difficult of detection. The other nodule showed strong Cu lines (0·002 %), a very strong Mn line (0·002 %), and traces of silver and lead. It is evident from the changes found here and in the aorta that the process of calcification provides a nidus for the deposition of the heavy metals. Inasmuch as copper and manganese were present in such large amounts, one would have expected iron to be well represented, but this was not the case. The iron spectrum was absent from the smaller specimen, and only showed faint indications in the larger one.

Neither specimen contained rubidium. The K lines were distinctly weak. The Ca lines were naturally very strong, and the antagonism that appears to exist between calcium and magnesium was also found here, the Mg lines being very weak, indicating the presence of less than 0·02 % of this element.

Table VII. *Lungs.*

Patient	Copper	Manganese	Rubidium	Silver	Other elements
S.	Trace	Trace	0·0024	Trace	—
F.M.	Trace	Trace	0·0024	Trace	—
K.T.	Trace	—	0·0016	Trace	—
A.B.	Trace	Trace	0·0024	Trace	—
G.E.	Trace	—	0·0016	—	—
N.C.	Trace	Trace	0·0016	—	Lead (trace)
E.B.	0·002	Trace	0·0016	Trace	—
Foetus D	Trace	—	0·006	—	—
Foetus (10)	0·002	—	—	—	Strontium (trace)
P.	Trace	—	0·0024	—	—
Foetus F	0·002	Trace	0·0024	—	—
G.P.	Trace	Trace	0·006	—	—

Lungs. A noteworthy feature of the lung spectra is the strength of the iron spectrum. The difficulty of washing this tissue free of blood does not account for the feature entirely, as in the transfused guinea-pig the iron lines of the lung were stronger than those of any of the other organs with the exception of the spleen. In E.B. and in P. (both of which were cases of silicosis, E.B. being a very advanced one) the iron spectrum was very rich, in the case of E.B. being exceptionally so. Indeed, in E.B. the Fe lines were stronger than those obtained by burning the same weight of dried normal blood-clot, indicating that the silicosis was accompanied by a definite siderosis. The Mg line was faint in both these specimens. Copper was present in all the specimens, but only in E.B. and the two foetuses did it rise to 0·002 %. Manganese, in traces only, was found in 7 of the 12 lungs. Rubidium was found in 11 specimens without great variation in quantity except in foetus D and the transfused guinea-pig, both of which showed high values. Faint traces of silver were found in five specimens, and traces of lead and strontium each in one case.

Table VIII. *Muscle.*

Patient	Copper	Manganese	Rubidium	Silver
M.R.	Trace	—	0·006	Trace
M.L.	Trace	—	0·006	Trace
Foetus F (pectoral)	0·002	Trace	0·0024	—
Foetus F (diaphragm)	Trace	Trace	0·003	—
Foetus D (quadriceps)	Trace (faint)	—	0·004	—
G.C.	Trace	—	0·006	—
R.M.	Trace	—	0·006	—

Muscle. The Mg lines are weaker in the foetal specimens (0·1 %) than in the adult (0·2 %). Copper was found in all cases in small amounts, but the Cu content of the full-term foetus shows one feature which may be of importance. Pieces were taken from the pectoralis major and the diaphragm, and there is a distinct difference in the copper contents of the two, that of the diaphragm being the lower. The foetus was stillborn and had never breathed, which suggests that the greater amount of copper in the pectoral muscle may be connected with its functional activity during the movements of intra-uterine life. In the adult muscles copper occurs in traces only, but all the organs are alike in showing a greater content of copper in the foetus than in the adult. Manganese was only found in the specimens from the full-term foetus. Rubidium occurred throughout, reaching a constant degree of concentration which is only equalled by the heart muscle and by the liver. Traces of silver were found in two of the specimens.

Calculi. Two calculi were examined, a single large cholesterol stone from the gall-bladder, and a small phosphatic calculus from the kidney.

The cholesterol stone contained the merest trace of magnesium, potassium, calcium, and iron, but a notable amount of copper was present (0·035 %). This figure agrees well with those found by Schonheimer and Oshima [1929] in their chemical analysis of six gall-stones, which varied between 0·016 % and 0·2 % Cu.

In the renal calculi the Ca lines were very strong, while both the K and Mg lines were weak. Manganese and rubidium were absent, but traces of silver and lead were found. There was only a faint indication of copper. The most remarkable feature is the presence of a well-marked Li line (0·0003 %). It is to be noted that the spectra of this and of the spleen of K.T. alone out of all those examined show a Li line. Some investigations now in progress, however, appear to show that this element is frequently found in the urine.

Cartilage. Five specimens of cartilage were examined. (Trachea from F.M., G.E.; rib cartilages from K.T., foetus D and foetus F.)

In G.E.—a man of 53—the K and Mg lines were weaker than in the others, while the Ca lines were stronger, presumably indicating a senile calcification. Copper was found in traces only in all the specimens except the full-term foetus (0·015 %). A trace of manganese was found in K.T. only. Rubidium occurred in all the specimens, the amount being smallest in G.E. while in the others it gave lines of approximately equal strength representing 0·003 %. A trace of lead was found in G.E. and the merest traces of iron were present in all five spectra.

Bone. (Parietal bone from K.T. and foetus D.) In both instances the weakness of the K and Mg lines and the strength of the Ca lines were the most prominent features of the spectra. Copper was just detectable, but manganese and rubidium were absent. The bone from K.T. contained a trace of lead.

Pituitary. (S.) Copper and manganese were both present in amounts of less than 0·002 % and 0·0008 % respectively. An infinitesimal trace of rubidium was found.

Brain. (Cerebral hemispheres S., K.T., foetus D., cerebellum K.T.)

The Mg lines were prominent throughout, and especially so in the case of the foetal brain (0·25 %). The Mg lines were stronger in the brain than in any other foetal organ. Copper was found in all the specimens, being strongest in the foetal brain (0·0035 %). Manganese was absent from all, save for a very faint trace in the cerebrum of K.T. Rubidium was found in all cases. Traces only were present in the brain of K.T. but in the cases of S. and foetus D this element reached values of 0·004 and 0·003 respectively. Comparing the cerebrum with the cerebellum of K.T., the former, in addition to containing a trace of manganese, was definitely richer in both copper and magnesium.

Teeth. Seven teeth were examined, one of which was an incisor of the first dentition. In the adult teeth, no differences were found between the incisors, molars, and bicuspids. The spectra showed the following features.

(*a*) A very low magnesium content, this line being only just visible. As would be expected the Ca lines were very strong, and were stronger in the crown of the tooth than in the fang.

(*b*) The teeth were rich in sodium, but were low in potassium. The K lines indicated a content of less than 0·08 %, as against about 0·6 % for an organ like the liver.

(*c*) The iron spectrum was so faint as to be almost invisible, but the lines due to copper were quite clear. It will have been noticed that all the foetal organs and tissues appear to be much richer in copper than the adult ones, and this difference applies equally to the teeth. In the deciduous tooth, the copper amounted almost to 0·002 %, while in the adult teeth, the lines, though clearly visible, were much weaker. Manganese was not found.

Milk. Five specimens of human milk were taken, but unfortunately the stage of lactation they represented was not noted. There was, however, no specimen of colostrum. All the milks showed lines due to sodium, potassium, magnesium, and calcium. There was no marked variation in these metals except that three of the milks were lower in both potassium and magnesium than the other two. Iron was found in minute traces in all. Copper was present in all the specimens, in amounts of less than 0·002 % of the dry weight, except in one instance, where it just reached this value. Traces of rubidium occurred in two out of the five specimens (0·001 %), but manganese was not found in any.

Carcinoma. Only one specimen of carcinoma has been examined in this series—a squamous-celled carcinoma of the oesophagus. Traces of copper and rubidium were found, but no manganese. The spectrum apart from these features, showed nothing unusual.

Blood. The results of the examination of the blood will be found in Table IX. In the case of blood and serum the examination was carried out on 0·1 cc., and in the case of the solid clot, on 0·05 g. of the dried material. No attempt has yet been made to examine the plasma as such, as it was feared that by the use of either vaselined tubes or citrate to prevent clotting, impurities might be introduced. The method adopted was to allow the blood to clot and the serum to separate. This was removed by pipette and centrifuged. The clot was then washed rapidly in distilled water and placed in the hot steam-oven.

In a few minutes a pool of serum was expressed from the clot. This was removed, the clot broken up and again rapidly washed in distilled water, before being finally dried. The method does not, of course, effect a complete separation of the clot from the serum, but it was thought best at this stage to employ it, rather than introduce the use of chemicals.

Table IX. *Blood.*

	Disease	Age	Sex	Copper	Manganese	Rubidium	Silver
1.	Anaemia of pregnancy	40	F.	0·002	—	—	Trace
2.	37 weeks pregnancy	—	F.	Trace	—	Trace	—
3.	Full-term ,,	—	F.	Trace	Trace	Trace	—
4.	36 weeks ,,	—	F.	Trace	—	Trace	Trace
5.	38 weeks ,,	—	F.	Trace	Trace	Trace	—
6.	12 weeks ,,	—	F.	Trace	Trace	Trace	—
7.	22 weeks ,,	—	F.	Trace	Trace	Trace	Trace
8.	Diffuse hyperplastic sclerosis	40	F.	Trace	—	—	—
9.	Myeloid leukaemia	?	F.	Trace	—	—	—
10.	Myeloid leukaemia. Clot	30	M.	Trace	—	0·006	—
11.	,, Serum			Trace +	Trace	—	—
12.	Foetus D. Heart blood (Dried whole blood)	—	—	Trace	—	0·004	—
13.	Foetus F. ,, (Dried whole blood)	—	—	0·005	—	0·004	—
14.	Foetus D (*a*). Liver blood (Dried whole blood)	—	—	0·005	—	0·002	—
15.	22 weeks pregnancy (clot)	—	F.	Trace	—	0·003	Trace
16.	,, (serum)	—	F.	Trace	Trace	—	—
17.	Paget's disease of bone (0·1 cc. blood)	25	M.	Trace	Trace	0·0016	—
18.	,, (serum)	25	M.	Trace +	Trace	—	—
19.	,, (clot)	25	M.	Trace	—	0·003	—
20.	Myxoedema	35	M.	Trace	—	Trace	Trace
21.	Carcinoma of ovary	28	F.	0·002	Trace	Trace	—
22.	Chlorosis	15	F.	0·002	Trace	Trace	—
23.	Tabes dorsalis	38	F.	Trace (faint)	Trace	—	—
24.	Cirrhosis of liver	63	F.	Trace (faint)	—	—	—
25.	Perinephric abscess	35	M.	Trace (faint)	—	—	—
26.	Pernicious anaemia	60	M.	Trace (faint)	Trace	—	—
27.	Polycystic kidney	36	M.	Trace (faint)	Trace	—	—
28.	Landry's paralysis	24	M.	Trace (faint)	Trace	—	—

The iron spectrum was strong in all the specimens of blood, with individual variations depending on the presence of anaemia. A very faint trace of iron was also seen in the centrifuged serum. Sodium appeared to be present in the serum in greater amount than in the clot. Potassium, on the other hand, was found chiefly in the red cells, the potassium spectrum of the serum being very weak. Copper occurred in all the specimens. It reached a strength that could be compared with the weakest standard solution in two of the three foetal cases, but only one of the adult bloods was sufficiently rich in copper to do this. It happened that this—a case of severe anaemia of pregnancy—was the first blood to be spectrographed, and the finding of an unusual amount of copper led to six other specimens of blood from cases of normal pregnancy being examined. The fact that the foetal tissues are so rich in copper, which must be supplied by the mother, gave rise to the expectation that pregnant blood might, in general, be characterised by such a richness in copper as was found in this case. The copper contents of the six pregnant bloods were higher than in the other adult bloods, but nowhere as high as in the anaemia of pregnancy. Evidently the large demands made by the foetus for copper cause this slight increase in the level of copper in the blood during pregnancy. The association in one case of a severe anaemia with an unusually high level of copper suggests that for some unknown reason there was an unusual mobilisation of the copper reserves of the mother, and our present knowledge of the relation between copper and the formation of haemoglobin suggests that it was the depletion of this which led to the anaemia.

In all the specimens examined, the copper appeared to be equally distributed through the serum and the clot. Manganese was found in eight of the specimens. In the three cases where clot and serum from the same blood were examined, the manganese was invariably found in the serum, but not in the clot. Rubidium, on the other hand, showed the reverse distribution, being found in the clot but not in the serum.

Traces of silver were found in the blood of five patients. It only happened to occur once in the cases where both clot and serum were examined, and then it was found in the clot, but not in the serum.

The bloods from myeloid leukaemia, myxoedema and diffuse hyperplastic sclerosis were not distinguished by any peculiar features. Eighteen sera which had been tested for the Wassermann reaction were examined, but no differences were noted between the positive and negative sera.

Lymph-gland. A gland from L. (lymphatic leukaemia) showed traces of copper and rubidium, but no other features of note. A partially calcified tuberculous gland from the mesentery of another patient, showed a trace of manganese in addition.

Bone-marrow. Marrow from the middle of the shaft of the femur of a man of 25, who died of miliary tuberculosis, was examined. The spectrum is poor in potassium, and neither manganese nor rubidium is present. Copper amounted to slightly less than 0·002 %.

Discussion.

The detailed description of the various human tissues shows that in addition to sodium, potassium, calcium, magnesium and iron, copper is a universal constituent of protoplasm, rubidium is almost universal, while manganese and silver, in addition to an apparently irregular distribution, are also constant constituents of certain organs. Each of these metals will be discussed separately.

Copper.

Every specimen taken from living tissue contains copper, though in a tissue such as bone the amount present is very small. The presence of copper in the tissues of plants and animals has been recognised for a long time, its occurrence being regarded as of either accidental or toxic nature, and it is only in recent years that the possibility of its being an element essential to life has had to be considered. The well-known work of Waddell *et al.* [1928, 1929], demonstrating the essential rôle played by copper in the formation of haemoglobin, is in keeping with the trend of other recent work which has demonstrated the widespread distribution of copper in living tissues [Lindow, Elvehjem and Peterson, 1929]. In a recent paper, Fox and Ramage [1931] found copper to be present in every one of 146 specimens of tissues removed from different species of invertebrates. In the present investigation, copper has been found in every one of 195 specimens of tissue removed from 23 human beings. The constant presence of copper in over 500 tissues from all parts of the animal and vegetable kingdoms, together with the evidence of McHargue [1925] and Maquenne and Demoussy [1920] as to its distribution in the plant world, leads to the conclusion that copper is a universal constituent of protoplasm. That it is also an essential constituent is rendered likely by its known importance in relation to haemoglobin formation in mammals and its occurrence in haemocyanin.

There are certain aspects of the distribution of copper in human tissues which merit separate attention.

(1) It is obvious that copper is of great importance in the functions of the liver during foetal life. The copper values in the five foetuses examined are between seven and ten times in excess of the adult percentage. This is in agreement with the finding of McHargue [1925] who in the liver of an ox found 50 mg. of copper per kg. of dry tissue, while in that of a calf born dead the figure was 908 mg. of copper per kg. It is probable that part at any rate of the peculiar importance of copper for the foetal liver may be connected with its blood-forming functions in this stage of life.

(2) The other foetal organs also contain a greater amount of copper than the corresponding adult tissues, though the difference is far less than in the case of the liver. This has been found by other workers. Thus Bodansky [1921] found that the copper content of the foetal brain is greater than that of the adult. Copper presumably plays an important part in the phenomena of growth. This seems to be the most feasible explanation of the difference in the copper contents of an incisor of the first dentition and of various teeth taken from adults. In view of the knowledge that is being accumulated by the work of Mellanby [1931] and others on the relation between an adequate vitamin supply and the formation of sound teeth, and the suggestion of McHargue [1925] that copper may play some part in the formation of certain of the vitamins, the relations that may exist between copper and the growth of the teeth merit further investigation.

(3) It is obvious that to supply the great demands of the foetus for copper, inroads must be made on the maternal store of this metal, unless a sufficient amount to meet the foetal demands is present in the food during pregnancy. The drain of copper from the mother does not cease after birth, for the examination of five specimens of human milk showed copper to be present in all. That it would be possible to take a diet deficient in copper is indicated by the investigation of Lindow, Elvehjem and Peterson [1929]. It therefore seems possible that the chronic secondary anaemia and general debility found

in some cases of pregnancy may be connected with a dietary deficiency of copper. The whole question of the metabolism of copper during pregnancy seems a promising field for investigation. Its probable importance is further suggested by the solitary observation that, of the pregnant bloods that were examined, the greatest amount of copper occurred in a case of severe secondary anaemia, which may have been due to some unusual demand of the foetus for copper leading to an abnormal mobilisation of the maternal store of this metal.

(4) Of the various diseases that were investigated, the only ones associated with marked abnormalities of copper content were those represented by R.M. (chronic interstitial nephritis) and J. (atrophic cirrhosis of the liver). In the absence of more extended knowledge, the meaning of the large amount (0·02 %) of copper in the livers of these cases must be left an open question.

(5) The presence of copper in bile (and in a gall-stone) suggests that this is a channel of excretion for the metal. In the case of two sheep that were examined, the copper lines were visible on burning 0·1 cc. of bile, while the average content of their gall-bladders (dry) was 0·005 %. The high figures also obtained in the human gall-bladders suggest that the body attempts to conserve its supply of copper by reabsorption through the wall of the gall-bladder, and this probably occurs through the intestine as well.

Manganese.

(1) Manganese is especially found in the liver, pancreas, kidney and suprarenal, and to a less extent in the heart and lung. Its distribution through the other tissues is irregular. This differs in several respects from the findings of Zbinden [1930], who obtained manganese only in the liver and kidney. Our results agree in showing that the liver contains the greatest amount of this metal.

(2) The high content in manganese of the tissues from the transfused guinea-pig is noteworthy.

(3) Of the human specimens, the greatest individual amounts are found in the tissues of S. All the organs contain normal amounts with the exception of a calcified nodule from the heart, the suprarenals, and the aorta. This man died from cerebral haemorrhage at the age of 77, and the unusually high amounts of manganese in the aorta and suprarenals especially, raises the interesting possibility that the development of arteriosclerosis may be connected with the metabolism of manganese.

(4) The examination of the liver of J. lends no support to the thesis that cirrhosis of the liver may be due to chronic manganese poisoning.

(5) Manganese was found in 14 out of 28 specimens of blood, its presence being confined to the serum.

Rubidium.

Rubidium lines were found in 16 of the 28 bloods examined. Rubidium is present in the cells, and not in the serum.

(1) Rubidium has a very much wider distribution than manganese in the tissues. This may be due to its greater diffusibility.

(2) The distribution of rubidium is, in general, the opposite of that of manganese. While the latter is chiefly found in the glandular organs such as the liver and pancreas, being present in traces in 7 only of the 12 hearts and in 1 only of the 6 specimens of muscle, rubidium reaches its greatest concentrations in the heart and striated muscle.

(3) The only tissue from which rubidium is absent in the adult is bone.

(4) The only individual to be without rubidium was the 10-weeks foetus. It is impossible to say whether this is a chance result, or due to the early age of the foetus, or to be associated with other mineral abnormalities that were present.

(5) In general, this element appears to be associated with potassium in its distribution through the tissues, the strength of the lines due to the two metals tending to vary harmoniously. It must be borne in mind, however, that the brightness of the lines of these two elements may not be entirely independent quantities, since Gooch and Phinney [1892] by eye-observations on low temperature flames have shown that the presence of potassium salts in minute quantities will materially increase the brightness of the rubidium spectrum, while the reverse occurs when the potassium is present in larger amount.

(6) Rubidium has been found in this investigation to be almost universally present in tissues. The fact that Zbinden [1930] makes no reference to it in the course of his work is probably due to the difference in technique—since he uses an arc spectrum while we employ a flame spectrum—and in a paper on milk he states that rubidium "ne donne pas de raies spectrales assez sensibles entre 2500 and 3500 Å."

The trend of previous work on the physiological behaviour of rubidium is to show that the metal can partially or completely replace potassium in Ringer's solution. Ringer himself [1883] noted this action, and it has been confirmed by Zwaardemaker [1919] and others. Loeb [1920] finds that rubidium chloride is able entirely to replace potassium chloride in the solution required for the development of the eggs of the sea-urchin into swimming blastulae. Mitchell *et al.* [1921] by perfusing frogs with Ringer's solution in which the potassium chloride was replaced by rubidium chloride found that the rubidium was taken up by muscles which had been kept in activity, but was not taken up by resting muscle. In feeding rats with rubidium, they found that the bulk of this metal was stored in the muscles, which is in agreement with the findings of the present paper. Mendel and Closson [1906], after the injection of rubidium salts into rabbits and cats, came to the same conclusion, stating that "muscles form the chief storehouse for the temporary retention of rubidium, in which they surpass the liver." That the inter-availability of potassium and rubidium in this way is not the whole story is shown by further experiments of Mitchell and Wilson who fed young white rats on a purified synthetic diet in which the salts of potassium were rep laced by those of rubidium. After 10 to 15 days the rats showed marked derangement, with irritability and tremblings, developing later into tetanic spasms, and death within 48 hours. In all these experiments it was also found that caesium behaved like rubidium in all respects. The physiological behaviour of both caesium and rubidium so far as it is known, appears to be identical, yet caesium has not been found in any of the present series of tissues, or in a series of examinations made by Fox and Ramage [1931] on animals of widely different genera. It is difficult to say whether this is due to some unknown difference between the physiological properties of the two elements or to the fact that caesium, though as widely distributed in nature as rubidium, is not present in such great amounts. In sea-water the proportion of caesium to rubidium is about 1:200. It is possible that it may be present in living tissues, but in such small amounts that very much larger quantities would have to be burnt than were employed here, in order to demonstrate its presence. The presence of rubidium in tissues has a further interest in that it also occurs in sea-water, the relationships between the mineral composition of blood-plasma and sea-water being a well-known phenomenon. Rubidium may prove to be of further importance in that it is a faintly radio-active element. In this respect it resembles potassium. Zwaardemaker [1919] finds that rubidium can replace potassium in Ringer's solution in proportion to their relative radio-active powers. Roffo and Landaburu [1925] find that the radio-activity of organs injected with rubidium chloride is variable, but that tumour tissue always shows a higher radio-activity than normal tissue, presumably owing to the fact that tumour tissue absorbs more rubidium.

Silver.

Silver is present in considerably less quantity than the preceding metals, never rising to 0·001 % of the dry weight. It was found in every specimen of thyroid and tonsil examined (8 in all). It was found in the other organs in the following percentage of cases: liver, 47 %; suprarenal, 44 %; lung, 36 %; muscle, 33 %; pancreas, 30 %; kidney, 23 %; heart, 14 %; spleen, 8 %; isolated instances occurred in the aorta and a calcareous nodule. It was absent from the rest of the tissues. In blood, traces of silver were found in 5 out of 28 cases. Its presence does not appear to be connected with age and is not universal, since it is absent from all the tissues examined from foetus D and from the case of R.M., though in neither of these were either the tonsils or thyroid examined. None of the guinea-pig specimens contained silver. Whether this element has any physiological function, or whether its presence is accidental, its irregular distribution being due to selective absorption by the different tissues, is uncertain. Zbinden [1930] using the arc spectrum has found silver in every human tissue he has examined, including blood; thyroid being especially rich in silver, with which these investigations are in agreement.

Lead.

This occurred only spasmodically. It was found in the liver, kidney and cartilage of G.E., the aorta and a calcareous nodule of S., the bone of K.T., the renal calculus and in the lung of N.C. This varied and irregular distribution strongly suggests that the metal is present accidentally.

Strontium.

This metal was found in the kidney of the guinea-pig, and in the liver and lung of the 10-weeks foetus. This scanty amount does not suggest that the metal has any physiological functions, and it has not been described by Zbinden [1930] as being found by his spectroscopic method. Some unpublished observations of ours, however, indicate that strontium is probably a constant constituent of the retina. It is curious that in the present investigation the 10-weeks foetus should provide two out of the three instances where this element occurs. The whole mineral content of this foetus appeared to be abnormal, being deficient in potassium, devoid of rubidium, and containing strontium and a high proportion of calcium. It would be interesting to enquire whether such early foetal death may, in other cases, be associated with abnormalities of their mineral constitution, or if the composition found is normal for that age.

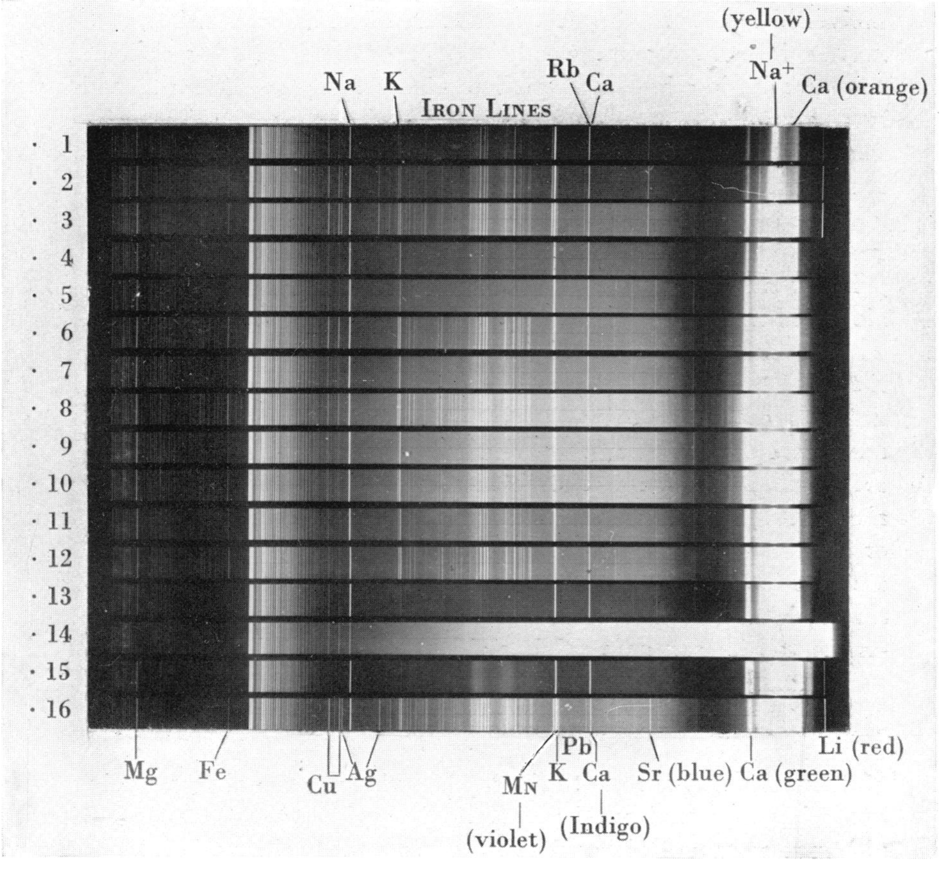
Lithium.

In view of its wide distribution through the animal kingdom, it was expected that this element would be of frequent occurrence, and its rarity came as a surprise. A lithium line was only found in two instances—a renal calculus and the spleen of K.T. Further work is required, since Hermann [quoted by Zbinden, 1930] states that it occurs in the foetus, and in the adult is concentrated particularly in the lungs, while Keilholz [1921] actually gives a table of values for the lithium content of the various organs. Zbinden [1930], however, makes no reference to lithium. In the course of some further work, we have found lithium to occur in human urine. It is possible that the differences which have been found are related to local variations in the composition of the soil or the water supply.

Summary.

1. Copper is a universal, and is probably an essential, constituent of living tissue. It occurs in greater concentration in foetal tissues than in adult, and reaches its maximum amount in the foetal liver, where the percentage of copper may be as much as ten times in excess of that present in the maternal liver.

2. Manganese occurs spasmodically in many tissues, but is chiefly concentrated in the liver, pancreas, suprarenal and kidney. It is present in bile, foetal bile containing a high proportion. In the blood it appears to be confined to the serum.



No. 1. 0·02 cc. standard solution.
2. 0·05 cc. standard solution.
3. 0·1 cc. standard solution.
4. Pancreas. Foetus D.
5. Gall-bladder and bile. Foetus D.
6. Liver. Foetus D.
7. Suprarenal. Foetus D.
8. Blood from liver. Foetus D.

No. 9. Kidney. Foetus D.
10. Spleen. Foetus D.
11. Quadriceps muscle. Foetus D.
12. Blood (clot). Myeloid leukaemia.
13. Blood (serum). Myeloid leukaemia.
14. Gall-stone.
15. Renal calculus.
16. 0·2 cc. standard solution.

3. Rubidium is almost as widely distributed as copper and occurs in greatest concentration in the heart and in striated muscle.

4. Silver is found to a less extent than the preceding metals, but appears to be a normal constituent of the thyroid and the tonsil.

5. Lead, strontium and lithium were only found to occur spasmodically.

It is hoped to pursue the various points that have been raised in the course of this investigation.

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