truly intracellular in the intact organisms, but have diffused through the membrane after the protoplasts were prepared, seems unlikely; although these compounds possess lower molecular weights than the nucleic acids (that from the streptococcus[6] has a mol. wt. of 14000), they do not dialyse through cellophane membranes and their shape and polyionic structure confer on them characteristics towards Sephadex gels normally associated with much larger molecules[4,6,10]. The presence of only small amounts of nucleic acid in the wall hydrolysate indicates that the membrane surrounding the protoplasts is an effective osmotic barrier.

The presence of small amounts of glycerol teichoic acid in the cytoplasmic contents could arise from incomplete separation of fractions, or could suggest either that these compounds are synthesised there or that certain cytoplasmic structures contain teichoic acid. The presence of traces of ribitol derivatives in the cytoplasmic contents of the streptococcus may be explained similarly.

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Microestimation of zinc in human blood serum

For the estimation of zinc in biological materials spectrography[1], polarography[2] or colorimetry of zinc dithizonate[3] is usually employed. For routine serial estimations the relatively simple colorimetric method appears to be the most suitable. However, dithizone yields coloured compounds with a number of other metals, so that the method is to be considered less specific and masking reagents must be employed. The purification of these reagents which usually contain considerable amounts of zinc causes difficulties.

The new method as proposed circumvents these difficulties by combining the colorimetry with chromatographic separation of zinc so that the masking reagents need not be used.

1 ml of blood serum was digested by the Kjeldahl method according to WOLFF[3]. The digest was then dried, dissolved in 1 ml three-times distilled water and transferred

quantitatively into an Emich's filtration flask where precipitation of heavy metals with 1 ml 0.1 N NaH_2PO_4 at about 100° took place. After cooling, the filtrate was separated and the sediment on the filter was washed three times. An aqueous solution of 2 N HCl was then added, so that the sediment was dissolved and poured into a vessel to dry. After drying the sample was ready for ascending chromatography on Whatman No. 1 paper in butanol saturated with 1 N HCl[4]. The chromatogram was dried in air at room temperature, then passed through 0.5 % 8-hydroxyquinoline[5] and saturated with ammonia vapour. The zinc spots characterized by a bright-yellow fluorescence in ultraviolet light were marked with pencil. After drying the spots were cut out and eluted with 10 ml 0.001 % dithizone in chloroform and kept in the dark for 20 min. The absorbancy of the sample was then measured against the dithizone solution. From the absorption curves of zinc dithizonate and of the free reagent (Fig. 1) is evident that a suitable wavelength is 505 mμ, where a large difference in the absorption of the two coloured solutions is found.

The calibration curve (Fig. 2) was obtained with known amounts of a zinc solution (250.0 mg of metallic zinc dissolved in the minimum amount of dil. H_2SO_4 and filled up to 500 ml with three-times distilled water) brought directly on the paper. The lowest molar ratio of dithizone to zinc was 2.55:1 (with 10 μg zinc). With higher amounts of zinc the quantity of dithizone should be raised.

The use of 8-hydroxyquinoline for the development of the chromatogram offers some advantages: in addition to making possible marking the spots in ultraviolet light, the solubility of the zinc oxinate in chloroform allows the reaction with dithizone to develop in one phase.

Since a large amount of alkali salts interferes by absorbing atmospheric moisture on the chromatogram, making a satisfactory separation impossible, the alkali metals are removed by the method described. It is advantageous to carry out all steps in

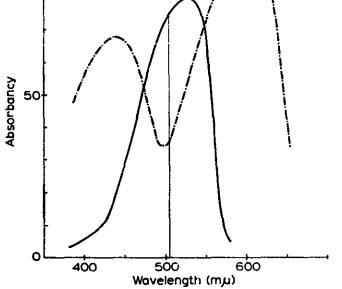

Fig. 1. Absorption spectra of zinc dithizonate (———) and of dithizone (–·—·–) in chloroform. Determined on Zeiss Universal Spectrophotometer, cell length 1.00 cm.

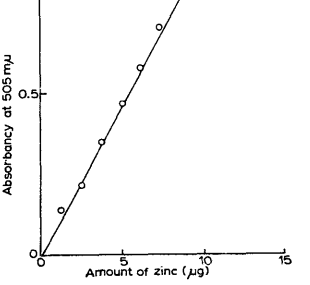

Fig. 2. Calibration curve, measured at 505 mμ, on Zeiss Universal Spectrophotometer, cell length 1.00 cm.

the same Emich filtration vessel. The other metals present in the blood serum (iron, copper and calcium)—even in excess—do not interfere with the determination.

All the chemicals must be of the highest purity, and aqueous solutions prepared with three-times distilled water and cleaned by shaking with dithizone by the usual manner. All glassware should be cleaned with nitric acid[6].

Since the dissociation constants of the two zinc dithizonate complexes are close to one another, the effect of the 8-hydroxyquinoline concentration on the production of the colour was observed[7] by adding gradually increasing amounts of 8-hydroxyquinoline to the spots on the chromatogram containing 10 μg zinc. From the results given in the Table I, is evident that the amount of 8-hydroxyquinoline does not influence the colour production.

TABLE I

EFFECT OF OXINE ON THE PRODUCTION OF ZINC DITHIZONATE

No.	*Zinc (μg)*	*Oxine (mg)*	*equiv of oxine: equiv of zinc*	*Absorbancy*	*Zn found (μg)*
1	10.0	0.25	5.63	1.09	10.6
2	10.0	0.50	11.2	1.06	10.2
3	10.0	1.00	22.5	1.06	10.2
4	10.0	1.50	33.7	1.04	10.0
5	10.0	2.00	44.9	1.07	10.4

TABLE II

ESTIMATION OF ZINC IN HUMAN SERUM

No.	*Serum (ml)*	*Zn added (μg)*	*Dithizone (ml)*	*Absorbancy*	*Zn found (μg)*	*μg Zn/ml of serum*
1	1.0	—	10.0	0.315	3.9	3.9
2	2.0	—	10.0	0.505	6.3	3.1
3	3.0	—	10.0	0.831	10.3	3.4
4	1.0	5.04	10.0	0.690	8.6	3.5
5	1.0	10.08	20.0	0.568	14.2	4.1

The method described was used to measure the zinc content of the blood serum of normal healthy persons (see Table II) as well as of patients suffering from seborrhoea before and after peroral administration of high dosis of $ZnCO_3$. Whereas the healthy individuals reacted to the administrations of $ZnCO_3$ by a large rise in the zinc level of the blood serum, in some seborrhoical individuals this rise was not observed. The clinical results are the subject of a separate publication.

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