

VITAMIN A, AS DETERMINED BY THE BLUE UNITS OF THE ANTIMONY TRICHLORIDE TEST, IN THE LIVERS OF MALNOURISHED CHILDREN

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THE livers used in this investigation were obtained from children of the indigent classes dying from various causes in the Colombo hospitals.

Carr and Price (1926) originally described a method of determining the amount of vitamin A by the use of antimony trichloride. A quantity of the liver, or other organ, to be tested was dried and extracted in a Soxhlet apparatus, and the test carried out with the extract. This is a tedious procedure when large numbers of tests are being done, and Moore (1930) modified it by dissolving the samples in 5 per cent caustic potash and shaking this up with a little alcohol, and extracting the fats and vitamin A by adding ether and shaking the mixture.

When working in a hot and damp climate special precautions must be taken to prevent the presence of moisture in the final extract.

The method which has been followed is that described by Davies (1933) with a few small modifications :—

Method

Five grammes of liver are weighed in a 50-c.cm. beaker and minced with scissors, 10 c.cm. of 5 per cent solution of KOH is added and the material is transferred to a 50-c.cm. conical flask. The mixture is digested in an oven at 100°C. until there is complete solution. It is poured into a 100-c.cm. graduated and stoppered funnel and 5 c.cm. of ethyl alcohol is added and well shaken, 50 c.cm. of ether is added and the shaking repeated. The layers are allowed to settle out and the aqueous layer is run off and discarded; 5 c.cm. of water is added and vigorously shaken with the other layer; the aqueous layer settles and is discarded; the washing to remove the KOH is completed by gentle agitation with 50 c.cm. of water (strong shaking produces an emulsion which will not readily settle into layers). The ether fraction is passed into a small flask containing anhydrous sodium sulphate and allowed to stand for a few minutes; this absorbs the water; the ether is filtered through a little more anhydrous sodium sulphate in a dried sintered glass funnel leading into 100-c.cm. wide-neck squat flask. The content of the flask is evaporated in a water bath, and, to prevent condensation of moisture, the flask is fitted with a cork through which two tubes pass—one leads to two wash bottles, the first contains sulphuric acid and the second caustic potash, and the other leads to a suction pump, thus only dried air enters the flask. The residue after evaporation should be a clear and transparent oil; it is dissolved in 2 c.cm. of dry chloroform; a blood pipette delivers 0.02 c.cm. of this solution into a 1 cm. Lovibund cell and the volume is made up to 0.5 c.cm. Two c.cm. of antimony trichloride in dry chloroform (Carr-Price reagent) is added from a B.D.H. automatic pipette; if a blue colour appears it is quickly matched in a Lovibund tintometer with blue and yellow glasses; similar tests are made until the content of the cell is matched by Lovibund blue units, and the necessary yellow glasses. The calculation is made as follows:—

$$\frac{\text{Five blue units} \times 2.5 \text{ volume of reaction mixture}}{\times (2) \text{ amount of chloroform.}}$$

$$\text{Amount giving 5 blue units} \times \text{grammes of liver (5).}$$

Supposing 0.2 c.cm. gave five blue units, then—

$$\frac{5 \times 2.5 \times 2}{0.2 \times 5} = 25 \text{ blue units per gramme of liver.}$$

When a large number of blue units is present it is necessary to dissolve the evaporated residue in a greater quantity of chloroform, such as 5 or 10 c.cm.

Most of the livers of the children were checked against rabbits and bullocks' livers. The former ranged from 95 to 220 blue units per gramme, whereas the latter ranged from 1,250 to 2,350 blue units per gramme. This is not an exact check but it serves as a fairly satisfactory control when there is little or no vitamin A in the livers tested.

Seventy-seven livers have been tested; four were from stillborn children and the others from children of various ages.

Stillborn children

The four stillborn children were from very anæmic mothers who were between the seventh and eighth months of pregnancy. The results were :—

Blue units in gramme of liver.	Mother's hæmoglobin index.
350	45 per cent
25	anæmia ++
40	50 per cent
5	45 per cent

This shows great variation in the amounts of vitamin A in the livers of those stillborn children; but there is no correlation between the blue units and the degree of anæmia of the mother.

Livers which showed the highest blue units

The livers of the 73 children were in most cases from children who had died within a few days of entering hospital. The livers which showed the highest blue unit values were :—

Age.	Sex.	Cause of death.	Blue units per gramme.
12	M.	Endocarditis	155
10	M.	Killed in motor accident.	125

The boy who died of endocarditis had been in hospital for some time and had been well fed. The boy killed in the motor accident appeared well nourished but he belonged to the working classes and possibly about 125 blue units may be taken as a standard for this class of persons in the tropics, who usually live on diets containing little or no vitamin A and not large quantities of its precursor.

Livers which showed no blue units

There were 15 livers in which not a trace of blue appeared in the mixture containing the extract from 2 grammes of liver, the maximum

amount used in these tests. The following table gives a few details of those :—

the amount of improvement the skin eruption had undergone during the stay in hospital.

*Livers showing no blue units in 2 grammes*

Number	Age	Sex	Clinical condition	Degree of emaciation	Length of stay in hospital
1	10 months	F.	Bronchitis, malnutrition with œdema	+	2 days
2	3 weeks	M.	'Congenital syphilis'. Mother's Wassermann ++.	+	2 "
3	8 years	F.	Nutritional œdema	?	2 "
4	1 year	F.	Colitis	+	7 "
5	4 weeks	F.	'Prematurity'	++	5 "
6	12 years	F.	'Phthisis with œdema of hands and feet	+	4 "
7	4 "	M.	Chronic diarrhoea	0	8 "
8	2 "	F.	Enteritis and marked phrynoderma	++	6 "
9	15 "	M.	Ankylostomiasis and anæmia	?	5 "
10	5 "	M.	Brain abscess	0	7 "
11	6 months	F.	Infantile debility	++	3 "
12	6 "	M.	Gastro-enteritis	++	4 "
13	17 years	F.	Phthisis	++	10 "
14	5 "	F.	Anæmia, keratomalacia and phrynoderma	+	4 "

It will be noticed that the livers of three children with œdema showed no blue units, two of these (numbers 1 and 3) were typical cases of nutritional œdema. The parents of number 6 were indigent and it is probable that she had been poorly fed.

There were four cases of 'enteritis' (numbers 4, 7, 8 and 12) and two of these, numbers 4 and 7, had been in hospital for a week or more and had received cod-liver oil and other substances rich in vitamin A; and it appears a justifiable conclusion that vitamin A is not readily absorbed from a disordered alimentary tract.

There were only two patients who are recorded as showing marked phrynoderma, namely numbers 8 and 15, and the latter patient had keratomalacia.

Two patients (numbers 9 and 11) might equally well have been diagnosed as suffering from malnutrition.

Thus in 11 out of the 15 patients there was definite evidence of malnutrition or defective absorption.

The diagnoses in the four remaining cases were congenital syphilis, prematurity, brain abscess and phthisis.

*Livers showing blue units*

It is the routine practice in the hospital, to which most of the children had been admitted, to give all patients adequate amounts of fish-liver oils, or other preparations rich in the fat-soluble vitamins; consequently positive antimony trichloride tests with the livers of children who have been in hospital for many days are of little or no significance except that it may indicate that satisfactory absorption has taken place.

Eight patients, who had varying degrees of phrynoderma, had livers which give various numbers of blue units per gramme, but unfortunately no records were kept which indicate

There was a patient, aged 7, who had phrynoderma and keratomalacia and the liver showed 35 blue units per gramme, but the child had been in hospital for 10 days and appears to have died of a terminal bronchitis, and during the stay in hospital had received considerable amounts of preparations containing vitamin A.

It is noteworthy that the only three cases of nutritional œdema in this series also showed an absence of blue units.

*Discussion*

Many of the poorest class children in Ceylon are weaned to a diet poor in proteins and deficient in all vitamins, and it is not astonishing that various signs and symptoms of malnutrition are prevalent among them. In many cases the deficient diets produce debility paving the way for infections which lead to death.

The œdemas of malnutrition are usually attributed to a deficiency of vitamin B, and in many cases there is also a deficiency of proteins, and probably this occurred in the three cases reported here, but the fact that vitamin A was also deficient without the presence of signs usually attributed to this deficiency serves to show that a human diet which is deficient in one respect is often deficient in many others; and further that the absence of any particular signs does not necessarily exclude any particular deficiency.

The results in cases of enteritis indicate a defective absorptive power of the alimentary tract for fat-soluble vitamins; this is in accordance with the general experience that fats are not well absorbed when there are digestive disturbances. Carotene, the precursor of vitamin A, may be more easily absorbed, and, if so, is indicated in cases of enteritis; this may explain the widespread use of extracts of leaves in diarrhoeas and dysenteries among the masses of the tropics.

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## A SYNCOPAL FORM OF ANGINA PECTORIS

(AN ELECTROCARDIOGRAPHICAL STUDY)

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GALLAVARDIN (1922) described a form of angina pectoris which was characterized by attacks of syncopal syndrome something like that of Adams-Stokes'. He considered these in relation to a temporary vagal inhibition which is exaggerated in this condition. Such cases are, however, very rarely met with. The following case therefore seems to be worth reporting :—

The patient was a retired clergyman, aged about 56 years. One afternoon at about 2 p.m., while attempting to catch a train for which he was waiting on the platform, he felt vertigo and giddiness and fell down unconscious. He remained in this condition for more than two hours. On admission into the hospital he was still unconscious and had a pulse rate of about 30 per minute and a blood pressure of about 98/60 mm. Hg. After atropine and cardiazol injections, he soon regained consciousness and began to complain of pain in his chest behind the sternum. The pulse rate rose to about 60 per minute. The blood pressure too showed a rise of about 130/90 mm. Hg. Five months ago, he said, he had a similar attack of momentary loss of consciousness which was overlooked at that time.

The general condition of the patient remained fairly good thereafter. The precordial pain was all that he complained of. The pain was located in the right side of the chest close behind the sternum. It came at intervals of about 15 to 20 minutes. It was of a spasmodic character, 'twisting' in nature. It had a tendency to radiate towards the suprasternal notch. The

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Cramer (1933) has reported some experiments on rats in which he has shown that when diets are deficient in vitamin B, there is defective absorption of vitamin A, and this may lead to xerophthalmia. This suggests that there are interactions among the vitamins, and whatever signs and symptoms may be present in all cases the correcting diets should be ample in all constituents.

### Summary

A short description is given of a method of determining the amount of vitamin A in livers by the antimony trichloride test.

There was great variation in the numbers of blue units shown with the livers of stillborn children.

Fifteen out of 63 livers showed no blue units.

Four of these livers were from children with 'enteritis' and seven from children showing other signs of malnutrition.

### REFERENCES

- Carr, F. H., and Price, E. A. (1926). *Biochem. Journ.*, Vol. XX, p. 497.  
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 Davies, A. W. (1933). *Biochem. Journ.*, Vol. XXVII, p. 1770.  
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characteristic radiation along the arm was absent. He did not feel any pain during sleep which was undisturbed owing to a soporific he was taking.

Physical examination revealed an emphysematous condition of the chest. The heart was more or less centrally placed. There was no apparent enlargement. Heart sounds were almost inaudible. There was no œdema or rise of temperature. Blood examination showed a slight degree of anæmia, hæmoglobin 50 per cent, and 2,900,000 red cells; the Wassermann reaction was negative. Biochemical examination gave a practically normal picture. Urine examination was negative. An x-ray examination of the chest showed no abnormality.

An electrocardiographical examination was undertaken on the third day after the attack. It revealed the cardiac nature of his troubles (*see later*).

As he showed no further alarming cardiovascular reactions, he was treated with injections of grape sugar to improve his cardiac condition, and he left the hospital after about a fortnight.

He was readmitted into the hospital after a fortnight with a similar history of another cardiac attack with loss of consciousness associated with convulsive movements of the limbs. This time too he complained of pain in the chest which was very similar to that described above. An electrocardiographical examination undertaken this time also revealed an exactly similar picture.

### Comment

The electrocardiograms were very peculiar and, from the point of view of diagnosis, of the utmost importance. The ventricular complexes in leads II and III were of abnormal amplitude, notched near the apex of the extremely deep S deflections and were also widened beyond 0.12 seconds. The T and the main ventricular deflections were in opposite direction with respect to each other. The S-T segments were well above the iso-electric line. In short, in these two leads the picture resembled very much a right-bundle-branch block. The lead I was, however, very atypical. The amplitude of excursions was very small. The complexes were widened and thickened and the T deflections were almost iso-electric. The whole picture taken together was thus atypical for a pure right-bundle-branch lesion.

Mahaim (1927) has drawn attention to the fact that pure cases of right-bundle-branch block may be comparatively rare to find in as much as a clean severance of the right-bundle branch is not usually seen in human pathology analogous to that in an experimental animal. More commonly, a mixed type of right-bundle-branch block may be obtained clinically. From an anatomical consideration this seems very reasonable, as will appear from the accompanying diagram. Mahaim has described two different types of right-bundle-branch lesions: 'Bloc mixte de la branche droite a type anterieur' and 'le bloc mixte de la branche droite a type posterieur' (a mixed anterior type and a mixed posterior type of right-bundle-branch block). These can be recognized in electrocardiograms. In the first case the amplitude of excursions is very high in leads II and III, and lead III may be small and atypical, while in