The following tests were made :

(1) Comparison of the activity of the plasma injected immediately after the ascorbic acid had been added with the same plasma not treated. *It was seen that the activity was not appreciably modified.*

(2) Comparison of the activity of the two fractions obtained from the plasma after treatment with ascorbic acid; kept in the ice-chest for three hours and centrifugal separation of the precipitate from the liquid above it. (The precipitate was washed on the surface with saline solution.)

Supernatant fluid				Precipitate	
Animals inoculated				Animals inoculated	
(1) died of erythroblastosis after	9	days		(1) survives	
(2) " " " " "	12	"		(2) "	
(3) " " " " "	14	"		(3) "	
(4) " " " " "	17	"		(4) "	

(3) Comparison of the activity of the fraction obtained after treatment of the plasma with ascorbic acid and elimination of the precipitate in the way mentioned above, with the same plasma not so treated.

Plasma (+ oxalate) not treated			Ascorbic acid plasma supernatant fluid		
Animals treated	Positives	Average time of survival of positives	Animals treated	Positives	Average time of survival of positives
21	15	19 days	21	19	15 days

It seems, therefore, that the activity of the plasma treated with ascorbic acid (contrary to the results obtained by Claude^{1,2} by acidification with other acids of watery extracts of Rous's sarcoma) remains altogether in the supernatant fluid; and indeed, the activity seems in some degree to be increased in respect to the normal plasma.

Other researches are being carried out with plasma of fowl affected by erythroblastosis brought, by means of lactic acid or galacturonic acid, to the same pH, so as to ascertain whether in reality the elimination of an inactive substance from the leukæmic plasma and its successive activation is, as would seem from purely preliminary observations, the consequence of an elective action on the part of the ascorbic acid itself.

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¹ Murphy, J., Sturm, E., Claude, A., and Helmer, O., *J. Exp. Med.*, **56**, 91 (1932).

² Claude, A., *J. Exp. Med.*, **61**, 27 (1935).

Role of Manganese in the Biological Synthesis of Ascorbic Acid

SPECULATION on a possible explanation of the difference in ascorbic acid contents of different plants led me to the idea that this difference may be due to the activating influence on the synthesis of ascorbic acid by plants of some mineral or minerals which are

not detectable by ordinary analytical methods. A close study of the paper by Boyd and De¹, who have made a spectrographic analysis of a number of vegetable and animal products, revealed the fact that the manganese lines are very prominent in those vegetable products and animal tissues which are generally rich in ascorbic acid content. This led me to the investigation of the role of manganese in the synthesis of ascorbic acid by plants and animals.

By germinating Bengal gram (*Cicer arietinum*) in distilled water and in dilute manganese sulphate solutions containing manganese equivalents of 1 in 10⁶, 1 in 10⁵ and 1 in 10⁴ for six days under identical and sterile conditions, it has been found that the ascorbic content of gram germinated in dilute manganese solutions is higher than that of gram germinated in distilled water, the respective amounts synthesized being 63.7 mgm., 75.5 mgm., 86.6 mgm. and 84.7 mgm. per 100 gm. of the original dry grams. The ascorbic acid assay was made by a modified Tillmann method after reducing the dehydroascorbic acid in the extracts with hydrogen sulphide. The germination was better in 1 in 10⁶ and 1 in 10⁵ manganese solutions and more stunted in 1 in 10⁴ manganese solution than in distilled water.

Fuller details of this and experiments on animals which are in progress will be published elsewhere.

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¹ Boyd, T. C., and De, N. K., *Ind. J. Med. Res.*, **21**, 109 (1933).

Absorption of Carotinoids from the Human Intestine

It is generally supposed that, for human beings, carotene of vegetable origin is the principal source for vitamin A. If this is true, it is important to know the absorbability of leaf carotene. We have

	Test dose		Excretion in faeces			
	carotene	xanthophyll	carotene	%	xanthophyll	%
50 gm cooked spinach	1080γ	3675γ	1017γ	94	2478γ	67
50 gm. cooked spinach + 30 gm. lard	1660	4805	1573	95	3893	81
100 gm. cooked carrots	5700	370	5643	99	163	44
β-carotene dissolved in oil	1056		437	41		

investigated this on two healthy male adults (namely, ourselves).

For one to two weeks a diet was followed which was very poor in carotinoids (bread, rice, lard, meat, Marmite and 50 mgm. ascorbic acid daily). Then the excretion of carotinoids in the faeces was determined before and after intake of a test dose of vegetables containing a known quantity of carotinoids. The faecal carotinoids were extracted with alcoholic potash, shaken out with peroxide-free ether and after evaporation of the ether dissolved in petroleum ether. The carotinoids were estimated colorimetrically with the Zeiss stepphotometer after chromatographic analysis according to Tswett with activated aluminium oxide (Brockmann).

While on this diet, 6-10γ carotene and 57γ xanthophyll per 20 gm. faeces were excreted. In the accompanying table the results are summarized of the determinations of the excretion above this level after intake of different test doses.