

fact that trypanosomes, which have been made completely resistant to the aromatic arsenicals and to Bayer 205, respectively, are very sensitive to undecane diamidine, and to synthalin.

#### SUMMARY

The investigation described in this paper was prompted by the discovery that synthalin has a direct trypanocidal action.

A considerable number of guanidines, isothiouras, amidines and amines, with alkyl and alkylene chains, were prepared and examined for trypanocidal activity. It was found that certain of the diamidines exhibit a powerful trypanocidal action in vitro. With the most active member of the series, undecane diamidine, it is possible to produce permanent cures in infected laboratory animals. As undecane diamidine is of entirely different chemical constitution from all known trypanocidal substances, this discovery is of considerable academic interest. This interest is increased by the fact that resistance to the drug is acquired by the trypanosome very slowly, if at all, and that trypanosomes which have been made completely resistant to the aromatic arsenicals and to Bayer 205 exhibit no resistance to this compound. It is also of practical significance in that it opens up a new field in the search for substances of therapeutic value against trypanosomal infections.

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## THE INADEQUACY OF SYNTHETIC ASCORBIC ACID AS AN ANTISCORBUTIC AGENT

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IN 1928-35 Szent-Györgyi demonstrated the presence of ascorbic acid in all antiscorbatic substances. Later, when it was found that the synthetically prepared ascorbic acid also had an antiscorbatic effect, the conclusion was generally accepted that synthetic ascorbic acid and vitamin C were absolutely identical. Recently, however, we have been impressed by the fact that some patients presenting typical prescorbutic and scorbutic conditions have not reacted to the administration of synthetic ascorbic acid in the manner that would be expected if this were the case.

As one of us (A. E.) has provided data (to be published later) concerning the absorption and elimination of ascorbic acid, this clinical observation was subjected to closer investigation. For a time the capillary resistance test of Göthlin was applied to all patients admitted to the service, and, in addition, the

serum ascorbic acid of these individuals was determined by the method of Lund and Lieck (1936). A total of 29 patients were found to present signs of hæmorrhagic diathesis together with a low ascorbic acid concentration in the serum, and each of them was given 300 mg. of ascorbic acid daily by mouth for ten days. In all but 3 this led to disappearance of the tendency to hæmorrhage and a return of the serum ascorbic acid to normal. (In passing, it may be observed that one patient, not included in this list, had to be given ascorbic acid by the intravenous route before he was cured.) Three patients, however, differed from the others in that the hæmorrhagic condition was not cured, and the serum ascorbic acid not increased by the oral administration of ascorbic acid. They had all received by mouth a total of 3 grammes, and they were now given an additional 300 mg. of ascorbic acid intravenously every day for ten days. But even then the hæmorrhagic condition was not abolished, nor was the serum ascorbic acid brought up to normal.

After these 3 patients had taken a total of 6 g. of ascorbic acid, they were given the juice of ten lemons every day for ten days. (By the Lund-Lieck method each lemon was found to contain from 20 to 40 mg. of ascorbic acid.) With this change in treatment the hæmorrhagic condition disappeared and the serum ascorbic acid returned to normal.

*Methods of analysis.*—The serum ascorbic acid was estimated by (a) the macro-method of Lund and Lieck (1936) and (b) the micro-method of Elmby and With (1937). The ascorbic acid in urine was estimated by the Lund-Lieck method (Dr. Lund kindly placed his method at our disposal before it was published, for which we are very grateful). We have also in some cases used Harris and Ray's method. All analyses were made in duplicate. The ascorbic acid in the serum is recorded as mg. per 100 c.cm. and the ascorbic acid in the urine as mg. The determinations in serum were performed daily.

Only some of the results obtained will be given here; a complete report will be published elsewhere.

#### THE THREE CASES

CASE I.—A tailor's apprentice aged 24. He had suffered from diarrhœa since the age of 7, the attacks lasting a month, with intervals of about a month. After he had reached the age of 11, blood and mucus were occasionally observed in the stools. His condition usually improved during summer and autumn, especially when he was able to get more fresh fruit. The disease was always at its worst during the winter. No bleeding from the gums, but he had a complete set of artificial teeth. He mentioned that he bruised easily.

The test-meal (Ewald) showed the quantity of free hydrochloric acid to be 50, and the total quantity of acid 80. Stools: blood ++, mucus +, pus ++ (quantitative test with catalase by the Thune-Andersen and Kemp method). Radiography: stomach, no abnormality; colon, typical findings of severe ulcerative colitis; long bones, some rarefaction. Blood picture: hæmoglobin, 94 per cent. (Haldane); white cells, 5080 (differential count normal); thrombocytes, 320,000 (O. Thomsen method).

The serum ascorbic acid at the beginning of the observations ranged from 0.02 mg. per 100 c.cm. to 0.03 mg. at the end of five days. During ten days' administration by mouth of 300 mg. of ascorbic acid daily the values remained at 0.02-0.03 mg., and when 300 mg. was injected intravenously each day they remained the same. During the administration of lemon juice the figures rose as shown in Table I.

After this the lemon juice was discontinued and the patient was given instead 300 mg. of ascorbic acid each day by mouth. The serum ascorbic acid then fell from 0.78 mg. per 100 c.cm. to 0.02 mg. in fifteen days, rising to 0.03 mg. in the next five days. Lemons were therefore again supplied, and their effect is seen in Table II.

Administration of lemon juice was then discontinued for thirty days, and 300 mg. of ascorbic acid was given by mouth for twenty days with the result that the values fell from 0.76 mg. to 0.02 mg. in fifteen days, rising to 0.03 mg. in the last five days. After this 500 mg. of

TABLE I

Date.	Lemons.	S.A.A.*	A.A.U.†	Göthlin : petechiæ.	Date.	Lemons.	S.A.A.*	A.A.U.†	Göthlin : petechiæ.
April 26	5	0.02	0	88	May 9	5	0.42	14	..
" 27	5	0.02	0	..	" 10	5	0.41	14	..
" 28	5	0.04	0	..	" 11	5	0.44	15	40
" 29	10	0.20	0	80	" 12	5	0.39	16	..
" 30	10	0.24	0	..	" 13	5	0.39	16	..
May 1	10	0.22	0	..	" 14	5	0.44	18	44
" 2	10	0.24	0	76	" 15	5	0.74	40	..
" 3	10	0.23	1	..	" 16	5	0.75	61	..
" 4	10	0.25	4	..	" 17	5	0.76	46	36
" 5	10	0.26	3	80	" 18	5	0.78	46	..
" 6	5	0.35	6	..	" 19	5	0.76	48	..
" 7	5	0.36	15	..	" 20	5	0.82	108	8
" 8	5	0.44	13	64	" 21	5	0.84	106	..

\* S.A.A. = Serum ascorbic acid (mg. per 100 c.cm.).  
 † A.A.U. = Ascorbic acid in urine (mg.).

ascorbic acid was given daily by mouth for ten days, but the serum ascorbic acid still did not rise above 0.03 mg. Finally, renewed administration of lemons resulted in the rise shown in Table III.

CASE 2.—A man aged 55. For fifteen years he had had dyspepsia manifested by delayed pain and hunger-pain, and he was admitted to the hospital suffering from melæna. He had furthermore presented symptoms of arterio-sclerotic heart disease and gingivitis and peri-odontitis.

Test-meal (Ewald): free acid, 50; total acid, 70. Radiography: of stomach, pylorogastritis, ? ulcer. Stools free from blood and mucus. Proctoscopy, no abnormality. Blood picture: hæmoglobin, 100 per cent. (Haldane); white cells, 6040 (differential count normal); thrombocytes, 350,000.

TABLE II

Date.	Lemons.	S.A.A.	A.A.U.	Göthlin : petechiæ.	Date.	Lemons.	S.A.A.	A.A.U.	Göthlin : petechiæ.
June 16	10	0.02	0	92	June 21	10	0.14	6	..
" 17	10	0.02	0	..	" 22	10	0.52	46	32
" 18	10	0.12	5	..	" 23	10	0.64	40	..
" 19	10	0.12	6	48	" 24	10	0.68	42	..
" 20	10	0.13	8	..	" 25	10	0.82	102	8

The serum ascorbic acid at the beginning of the observation period ranged from 0.02 to 0.03 mg. per 100 c.cm. (five days). With oral administration of 300 mg. of ascorbic acid daily for ten days it rose to 0.04 mg. During this period no ascorbic acid was found in the urine. After this, 300 mg. ascorbic acid was given intravenously daily for ten days, and the ascorbic acid rose in the serum to 0.08 mg. per 100 c.cm. During this period the patient excreted 40-180 mg. in the urine. The substitution of lemons for the synthetic substance for ten days gave the results set out in Table IV.

CASE 3.—Nurse 44 years old. For twelve years she had suffered from dyspepsia, and she also complained of

TABLE III

Date.	Lemons.	S.A.A.	A.A.U.	Göthlin : petechiæ.	Date.	Lemons.	S.A.A.	A.A.U.	Göthlin : petechiæ.
July 29	10	0.02	0	100	August 1	10	0.18	6	..
" 30	10	0.02	0	..	" 2	10	0.48	38	..
" 31	10	0.12	3	40	" 3	10	0.58	40	8

diarrhœa with pain and the passage of a considerable amount of blood and mucus.

Mouth normal. Stools: blood +, mucus +, pus +. Radiography: no abnormality of stomach or intestine. Proctoscopy: mucous membrane œdematous; many bleeding points seen. Blood picture: hæmoglobin, 88 per cent. (Haldane); white cells, 6120 (differential count normal); thrombocytes, 440,000.

TABLE IV

Date.	Lemons.	S.A.A.	A.A.U.	Göthlin : petechiæ.	Date.	Lemons.	S.A.A.	A.A.U.	Göthlin : petechiæ.
June 26	10	0.08	28	30	July 2	10	0.56	40	14
" 27	10	0.09	11	..	" 3	10	0.59	32	..
" 28	10	0.21	16	..	" 4	10	0.67	36	..
" 29	10	0.48	19	20	" 5	10	0.69	38	2
" 30	10	0.46	18	..	" 6	10	0.69	96	..
July 1	10	0.52	28	..					

During five days' observation the serum ascorbic acid ranged from 0.02 mg. per 100 c.cm. to 0.025 mg. During ten days' oral administration of ascorbic acid it did not rise above this level, and the patient did not excrete any ascorbic acid in the urine, but after ten days' intravenous administration of ascorbic acid it reached 0.08 mg. During the latter period 40-180 mg. was excreted in the

TABLE V

Date.	Lemons.	S.A.A.	A.A.U.	Göthlin : petechiæ.	Date.	Lemons.	S.A.A.	A.A.U.	Göthlin : petechiæ.
June 17	8	0.09	0	153	June 20	8½	0.37	19	22
" 18	9	0.22	17	..	" 21	9½	0.48	36	..
" 19	9	0.30	21	..	" 22	8¼	0.66	82	4

urine daily, and after four days' interval, it was found to be 0.09 mg. In the next six days she was given a total amount of 52 lemons with the result seen in Table V.

CONCLUSION

The 3 cases described had the following characteristics in common: (1) a history of intestinal trouble extending over many years; (2) inability to absorb ascorbic acid, or to retain it after intravenous injection; and (3) cure of both humoral and clinical abnormalities following the administration of lemons.

The explanation we suggest is that for the absorption and retention of ascorbic acid some unknown substance (a co-vitamin) is required. This substance is present in lemons.

This unknown factor may either be part of some other foodstuff (in which case its absorption may be inhibited by certain intestinal diseases), or else it

may be produced in the intestine under normal conditions.

The hypothesis that this factor is the same as the P vitamin found by Szent-Györgyi is being investigated at present by one of us (A. E.).

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## STERNAL PUNCTURE IN PERNICIOUS AND ACHRESTIC ANÆMIA

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APLASTIC anæmia of the idiopathic type is a rare disease characterised by diminution of all the formed elements of the blood. When Ehrlich (1888) described the first case, showing aplastic and fatty marrow, which adequately explained the blood picture, aplasia of the bone-marrow was fixed as the criterion of aplastic anæmia and cases which conformed with this description continued to be collected and reviewed (Cabot 1908, Hirschfeld 1911, Smith 1919). It was found, however, that some patients that have the clinical appearance and blood picture of aplastic anæmia show a red hyperplastic, not fatty aplastic, marrow and on account of this discrepancy these cases were called pseudo-aplastic anæmia. Recently more attention has been drawn to this form, which seems to be the commonest (Thomson, Richter, and Edsall 1934). Wilkinson and Israels (1935 and 1936) described a special group of cases characterised by hyperchromia, normal or almost normal gastric acidity, no disturbance of the gastrointestinal tract or central nervous system, no pyrexia or evidence of hæmolysis, and a lack of, or only a poor response to, the pernicious anæmia factor. Because of their hæmatological similarity they believed that they were dyshæmopoietic anæmias of the pernicious type, and as the hæmopoietic factor is absent from the liver in pernicious anæmia but present in normal quantity in these cases, they believed that the patients were unable to utilise or mobilise that factor and therefore called them "achrestic anæmia."

## APPEARANCES IN MARROW

We have recently had at St. Bartholomew's Hospital a few cases which conformed with the description of this achrestic type, and to elucidate their true ætiology their bone-marrow as well as that of pernicious anæmia was studied with the aid of sternal puncture. I examined 9 cases of pernicious anæmia, 7 in relapse and 2 in remission, and 6 cases of aplastic anæmia, of which 4 were of the achrestic type. The white picture of the marrow in the achrestic type was more or less hypoplastic and did not differ materially from that of pernicious anæmia. It is therefore omitted here and only the erythropoiesis that is the centre of interest in both groups is

discussed. Findings of all the cases are arranged for the sake of comparison in the Table. The picture of the bone-marrow in a case of pernicious anæmia in relapse and in achrestic anæmia will now be described.

Table showing Sternal Puncture Findings

Case.	Hb. per cent.	Red cells (per c.mm.).	Megalo-	Macro-	Normo-
			blasts.	blasts.	blasts.
Percentage in marrow films.					
Average normal	100	5,000,000	0	0	13
PERNICIOUS ANÆMIA					
1	50	1,360,000	56.5	5.5	5.5
2	46	1,480,000	28	3	7
3	41	1,360,000	64	10	1
4	67	2,130,000	40	15	2
5	45	2,030,000	54	11	6
6	71	3,760,000	50	4	2
7	48	1,440,000	33	8	12
Average	..	..	46.5	8.1	5.1
ACHRESTIC ANÆMIA					
8	59	2,230,000	7	32	25
9	90	3,600,000	0	3	18
10	38	1,625,000	1	25	26
11	41	1,040,000	0	32	4
12	41	1,860,000	0	26	14
Average	..	..	0.3	27.6	14.6

Remarks.—Cases 1 to 7 in relapse; Cases 8 and 9 had liver before admission.

*Pernicious anæmia.*—Nucleated red cells were present in enormous numbers, often sticking to one another in the form of small islets at the periphery and tail of the film. They were mostly three to four times the size of normocytes, and had basophilic or polychromatic cytoplasm and large finely reticulated nuclei. The diameter of the nucleus rarely exceeded half the area of the cell and there was always a generous margin of cytoplasm. Fully hæmoglobiniferous forms were very few in number and their nuclei were smaller, more coarsely reticulated, and deeply staining. Some of these cells were very large with a deeply basophilic cytoplasm, and not infrequently a few nucleoli among their very fine chromatin meshwork (gigantoblasts or preme-galoblasts). In the larger forms the nucleus was always less mature than the cytoplasm, and reticular nuclei might be seen in cells which contained almost a full complement of hæmoglobin. Smaller forms, even normoblasts, were present, their cytoplasm polychromatic or red and their nuclei quite pyknotic.

*Achrestic anæmia.*—The bone-marrow, though hyperplastic, was distinctly less cellular than in pernicious anæmia, but there were many nucleated red cells which were sometimes in small groups. These cells, on the whole, were large but they never equalled in size the gigantoblasts of pernicious anæmia. They had basophilic cytoplasm and large nuclei which occupied most of the cell area. Nucleoli were sometimes met with, but the chromatin of the nucleus was more diffuse and arranged in coarse bands. Pyknosis of the nucleus was commonly in advance of hæmoglobinisation of the cytoplasm, and cartwheel arrangement of the chromatin, common to most macroblasts and normoblasts, was frequently seen. Many typical normoblasts were present. Only very few of the large cells were difficult to differentiate from megaloblasts and were thus grouped as such. Mitotic figures were occasionally encountered, but were less numerous than in pernicious anæmia.

## DISCUSSION

The definition of the terms megaloblast and macroblast, which are sometimes confused and improperly