

sor A. N. Shaw. Dr. J. A. Pearce and E. C. Walker reported the orbital elements of Lambda Andromedae based on a series of measurements made on high dispersion spectrograms taken at Victoria. Dr. E. C. Beals discussed results which indicate that some new molecular absorption lines recently discovered show characteristics closely similar to atomic lines. It is suggested that their probable origin is to be found in the solid particles responsible for general absorption in interstellar space and that laboratory investigations of the absorption spectra of such particles as are likely to be present in interstellar space might be fruitful in their identification. A new mechanical height computer for radiosonde observations was described and shown by Dr. W. E. Knowles Middleton.

In Section IV, Dr. W. A. Bell's presidential address dealt with the use of some floras in Canadian stratigraphy. Fossil floras have proved very useful in subdividing the very thick Carboniferous sediments of the Maritime Provinces into six groups, of which three are Mississippian and three Pennsylvanian. The use of the terms Mississippian and Pennsylvanian is more appropriate as regards major floral and tectonic events of the Acadian province than Lower and Upper Carboniferous. The former terms are not synonymous with the latter, for the Mississippian terminated in an early part of Upper Carboniferous time, as in the Mississippian valley region. The group subdivision, established mainly on floral evidence, is apparently the most natural one, for it is corroborated by tectonic events. Coal formation was not confined to one age as formerly assumed, but took place locally in the Pennsylvanian in each of the three ages represented by the groups of strata.

In Western Canada stratigraphic work of Dr. F. H. McLearn made possible the recognition of a sequence of three fossil floras in the Lower Cretaceous, respectively of Barremian (and? Neocomian), Aptian and Albion ages. Coals were deposited in Barremian time in southern Alberta and the Yukon, in Aptian time in northern Alberta and central British Columbia.

Nineteen other papers on geological and mineralogical researches were presented. Dr. Madeleine Fritz, of the Royal Ontario Museum of Paleontology, reported the recent discovery of the bryozoan species

*Trachytoechus moniliformis* Fritz, n. sp., in the Gaspé sandstone of Lemieux Township, Gaspé County, in the interior of the Gaspé peninsula. This has provided evidence to substantiate the belief that the rocks in which the specimen was found are of Middle Devonian age. Dr. F. J. Alcock, of the Geological Survey, Ottawa, presented evidence based on the findings of several striated surfaces and many erratics in central Gaspé which support his already published conclusions that the Labrador ice sheet crossed the Shick-shock Mountains.

Dr. H. S. Jackson, of the University of Toronto, president of Section V, spoke on "Life Cycles and Phylogeny in the Higher Fungi." The discussion centered in a comparison of life cycles in the rusts with those in the red algae. It was shown that not only do the normal cycles correspond very closely but that the same sort of simplified cycles occur in both groups. A life cycle comparable to that of the ascomycetes also occurs among the simplified red algae.

Professor Velyien Henderson, the Flavell Medal winner, presented an invitation paper entitled "Studies in Anesthesia with the Cyclopropane Group." Professor A. T. Cameron, of the University of Manitoba, outlined the results of his researches on the relative sweetness of certain sugars and mixtures of sugar. If a solution contains known concentrations of two or more sugars, a means has been found for calculating the sweetness of this mixture in terms of that of a specific concentration of sucrose or of glucose. It has been demonstrated that the sweetness of 25 per cent. sucrose is not more than (and is probably less than) 3.3 times that of 5 per cent. sucrose.

Forty-three other papers on various phases of biological and medical sciences made up the program of Section V.

Officers elected for the coming year were: *President*, Professor J. K. Robertson, Queens University, Kingston; *Vice-president*, Professor E. S. Moore, University of Toronto; *President of Section III*, Dr. J. A. Pearce, Dominion Astrophysical Laboratory, Victoria, B. C.; *President of Section IV*, Dr. J. S. DeLury, University of Manitoba; *President of Section V*, Dr. Robert Newton, president of the University of Alberta.

J. R. DYMOND

## SPECIAL ARTICLES

### EXTRINSIC FACTOR IN PERNICIOUS ANEMIA: INEFFECTIVENESS OF PURIFIED CASEIN AND OF IDENTIFIED COMPONENTS OF THE VITAMIN B COMPLEX<sup>1,2</sup>

WHEN beef muscle together with normal human gastric juice is administered daily to suitable patients

<sup>1</sup> From the Thorndike Memorial Laboratory, Second and

with Addisonian pernicious anemia, a hematopoietic response appears within 10 days.<sup>3</sup> Like beef muscle, several sources of the vitamin B complex, such as milk,<sup>4</sup> eggs,<sup>5,6</sup> liver,<sup>7</sup> yeast,<sup>8</sup> rice polishings and wheat germ,<sup>9</sup> contain the so-called extrinsic factor for the

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hematopoietic reaction with normal human gastric juice (intrinsic factor). Observations yet to be published indicate that the active material can be removed from beef muscle by repeated extraction with dilute acetic acid and that it is resistant to autoclaving and to alkalization. Likewise, as shown below, extrinsic factor can be partially or completely removed from crude casein by repeated precipitation or by extraction with dilute acid or with alcohol. Such procedures tend also to remove the known members of the vitamin B complex.

The lack of potency as extrinsic factor of riboflavin<sup>10</sup> and nicotinic acid<sup>11</sup> has already been reported. In the present study it was proposed to test additively the extrinsic factor activity of all members of the

Observations were made on a series of ten patients with typical Addisonian pernicious anemia. A full description has been given elsewhere<sup>13</sup> of the hematological methods and the interpretation of results, especially of reticulocyte responses,<sup>14</sup> as well as of the restrictions on the character and hours of administration of the basal diet. The various types of casein and certain of the vitamins tested were kept in dry form until just before being given to the patient. Biotin was dissolved in 30 per cent. alcohol, and choline hydrochloride, xanthopterin and folic acid in small amounts of water. These and the folic acid concentrate employed in Case 100 were kept in the ice-box as separate solutions until immediately before use.

The results shown in Table 1 apparently demon-

TABLE 1

EFFECT OF DAILY ADMINISTRATION OF VARIOUS TYPES OF CASEIN TOGETHER WITH 150 CC OF NEUTRALIZED NORMAL HUMAN GASTRIC JUICE DURING A 10-DAY PERIOD

Type of casein	Case 91	Case 92	Case 93	Case 94	Case 95
			<i>grams daily</i>		
"Washed" (A. H. Thomas)	50	50			
"Water soluble vitamin-free" (Harris)*	..	..	50	50	50
Alcohol extracted†	..	..	..	..	..
Initial R.B.C. (mils./cu.mm.)	1.53	2.56	1.07	1.44	1.88
Reticulocyte peak (per cent.)	15.0	7.5	10.8	5.6	2.4
<i>Interpretation</i>	pos.	pos.	pos.	weak pos.	neg. ‡

\* Exhaustively extracted with dilute acids during manufacture.

† "Washed casein" (A. H. Thomas) was extracted with cold 65 per cent. alcohol five times, then once with hot 95 per cent. alcohol.

‡ In a second 10-day period during which 30 grams of Ventriculin N.N.R. were administered daily, this patient showed a reticulocyte response reaching a peak of 24.8 per cent. from an initial red blood cell level of 1.34 mils./cu.mm.

vitamin B complex and of certain other accessory nutritional factors as they became available in pure form.<sup>12</sup> It was planned to administer simultaneously casein rendered free of extrinsic factor, on the theoretical basis that one or more of the vitamins, even if not effective independently, might become active as a prosthetic group on the casein molecule as a result of the action of the gastric juice.

strate that the daily administration of 50 grams of A. H. Thomas "washed casein" (Cases 91 and 92), or of Harris "water-soluble vitamin-free casein" (Cases 93 and 94), simultaneously with 150 cc of normal human gastric juice gave rise to moderately positive hematopoietic effects. However, after A. H. Thomas "washed casein" was extracted five times with cold 65 per cent. alcohol and once with boiling 95 per cent. alcohol, it no longer possessed extrinsic factor activity (Case 95). Borden "Labco vitamin-free casein" (Table 2, Cases 99 and 100) was also found to be ineffective. The hematopoietic activity noted when one of the pure vitamin mixtures was given in combination with Harris "water soluble vitamin-free casein" (Table 2, Case 98) resembles that seen fol-

<sup>2</sup> The expenses of this investigation were defrayed in part by the J. K. Lilly gift to the Harvard Medical School.

<sup>3</sup> W. B. Castle and W. C. Townsend, *Am. Jour. Med. Sci.*, 178: 764-777, 1929.

<sup>4</sup> F. R. Miller and W. H. Pritchard, *Proc. Soc. Exp. Biol. and Med.*, 37: 149-152, 1937.

<sup>5</sup> K. Singer, *Wien. klin. Wchnschr.*, 45: 1063-1064, 1932.

<sup>6</sup> D. K. Miller and C. P. Rhoads, *New Eng. Jour. Med.*, 211: 921-924, 1934.

<sup>7</sup> F. Reimann, *Med. Klin.*, 1: 880-881, 1931.

<sup>8</sup> M. B. Strauss and W. B. Castle, *New Eng. Jour. Med.*, 207: 55-59, 1932.

<sup>9</sup> W. B. Castle, *Ann. Int. Med.*, 7: 2-5, 1933.

<sup>10</sup> F. Diehl and J. Kühnau, *Deutsches Arch. f. klin. Med.*, 176: 149-153, 1934.

<sup>11</sup> C. Faarup and A. S. Ohlsen, *Ugesk. f. laeger*, 102: 905-906, 1940.

<sup>12</sup> Since the completion of the present observations we have learned by personal communications of the negative

results of certain tests for extrinsic factor activity in pernicious anemia which have been made by other investigators: A. D. Welch, C. V. Moore and L. D. Wright: Xanthopterin, folic acid concentrate and "known crystalline B vitamins except folic acid." R. W. Vilter and T. D. Spies: Inositol, folic acid concentrate, pyridoxine and adenylic acid.

<sup>13</sup> W. B. Castle and T. H. Ham, *Jour. Am. Med. Assn.*, 107: 1456-1463, 1936.

<sup>14</sup> G. R. Minot and W. B. Castle, *Lancet*, 2: 319-330, 1935.

TABLE 2  
EFFECT OF DAILY ADMINISTRATION OF VARIOUS SUBSTANCES TOGETHER WITH 150 CC OF NEUTRALIZED NORMAL HUMAN GASTRIC JUICE

Substances	Case 96	Case 97	Case 98	Case 99	Case 100
<i>First Period—10 Days</i>					
<i>Casein</i>			<i>grams daily</i>		
"Water soluble vitamin-free" (Harris)*	..	..	50	..	..
Alcohol extracted†	50	50	..	..	..
"Vitamin free—Labco" (Borden)‡	..	..	..	50	50
<i>Accessory Factors§</i>					
Thiamin	0.1	0.1	0.1	0.1	0.1
Riboflavin	0.1	0.025	0.025	0.1	0.1
Niacinamide	0.1	0.1	0.2	0.2	0.2
Pyridoxine hydrochloride	0.1	0.1	0.1	0.1	0.1
d-Calcium pantothenate	..	0.1	0.1	0.1	0.1
p-Aminobenzoic acid	..	..	2.0	2.0	2.0
Choline hydrochloride	..	..	0.3	0.3	0.3
i-Inositol	..	..	0.2	0.2	0.2
Biotin	..	..	..	0.002	0.002
Xanthopterin	..	..	..	0.009	0.005
Folic acid	..	..	..	0.0036	0.0023
Initial R.B.C. (mils./cu.mm.)	1.56	1.79	1.73	1.17	1.50
Reticulocyte peak (per cent.)	1.4	2.2	6.4	1.9	1.0
<i>Interpretation</i>	neg.	neg.	pos.	neg.	neg.
<i>Second Period—10 Days</i>					
<i>Beef muscle</i>			<i>grams daily</i>		
Meat extract ¶	..	..	..	200	..
Ventriculin N.N.R. (without gastric juice)	30	30	30	..	35 cc.
Initial R.B.C. (mils./cu.mm.)	1.66	1.56	1.80	1.54	1.79
Reticulocyte peak (per cent.)	17.2	11.2	7.4	13.6	8.9
<i>Interpretation</i>	pos.	pos.	second pos.	pos.	pos.

\* Exhaustively extracted with dilute acids during manufacture.

† "Washed casein" (A. H. Thomas) was extracted with cold 65 per cent. alcohol five times, then once with hot 95 per cent. alcohol.

‡ Repeated isoelectric precipitation in progressively more dilute solutions of sodium chloride during manufacture.

§ Folic acid and folic acid concentrate (Case 100), both prepared by fermentation methods, and xanthopterin were obtained through the courtesy of Dr. Y. SubbaRow, Lederle Laboratories, Pearl River, N. Y.; other accessory factors (except p-aminobenzoic acid) through the courtesy of Dr. D. F. Robertson, Merck and Company, Rahway, N. J.

¶ Courtesy of Mr. Braxton Valentine, Valentine's Meat-Juice Company, Richmond, Va. (35 cc are derived from 600 grams of lean beef).

lowing administration of this type of casein alone (Table 1, Cases 93 and 94). This positive effect is, therefore, probably attributable to the impurity of the casein. The pure vitamin mixtures were inactive when administered in combination with other types of casein (Table 2, Cases 96, 97, 99 and 100).

These observations suggest: (1) that the careful purification required to render crude casein "vitamin free" is also essential for the elimination of the extrinsic factor; (2) that a combination of casein so extracted with the pure accessory factors used and in the dosage indicated did not reconstitute the extrinsic factor activity of the crude casein; and (3) that, nevertheless, it is reasonable to continue to regard the extrinsic factor as a thermostable component of the vitamin B complex as yet unidentified.<sup>8,15</sup>

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<sup>15</sup> R. R. Williams and T. D. Spies, "Vitamin B<sub>1</sub> (Thiamin) and Its Use in Medicine," p. 134. New York: Macmillan Company, 1938.

## PHOTOSENSITIVITY AS A CAUSE OF FALSELY POSITIVE CEPHALIN-CHOLESTEROL FLOCCULATION TESTS<sup>1</sup>

THE cephalin-cholesterol flocculation test has been proposed by Hanger<sup>2</sup> as a method of detecting active hepatic disease. Others<sup>3,4,5</sup> have reported that normal individuals showed positive reactions of varying degree and frequency. Our use of this test has also been complicated by the frequent yet irregular occurrence of falsely positive reactions of the 2 and 3 plus grade. A puzzling feature was the fact that sera giving falsely positive reactions in one laboratory consistently gave negative reactions when tested in a

<sup>1</sup> This investigation was conducted under the Commission on Measles and Mumps, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, U. S. Army, Washington, D. C. The studies were done at the Medical Clinic, Hospital of the University of Pennsylvania, and at the Biochemical Laboratory, Philadelphia General Hospital.

<sup>2</sup> F. M. Hanger, *Jour. Clin. Investigation*, 18: 261, 1939.

<sup>3</sup> F. J. Pohle and J. K. Stewart, *Jour. Clin. Investigation*, 20: 241, 1941.

<sup>4</sup> J. G. Mateer, J. I. Baltz, P. F. Marion and J. M. MacMillan, *Jour. Am. Med. Assn.*, 121: 723, 1943, Postscript.

<sup>5</sup> J. W. Oliphant, A. G. Gilliam and C. L. Larson, *Pub. Health Rep.*, 58: 1233, 1943.