his co-workers, among which mention may be made of one in which the isolation of some new sulfamides in the 1-1-Diphenylindane was announced. Perhaps the most interesting paper presented to the section was one of those given by Professor E. F. Burton, in which J. H. L. Watson showed how stereoscopic photographs had been taken with the electron microscope and exhibited several examples to the members present. This new technique enables one to view the objects photographed so as to bring them out in three dimensions, thus adding depth to the pictures and revealing new features in the structure of smoke and metallic particles. At the conclusion of the sectional meetings, Professor T. Thorvaldson was elected president of the section for the year 1943–44.

In Section IV (Geological Sciences), Professor M. B. Baker gave the presidential address and spoke on "Gold and Iron Prospects in Canada." This address was followed by sixteen papers on various geological investigations. Among these Dr. E. A. Hodgson delivered a very interesting paper on the "Rock Burst Experiments at Lake Shore Mines, Kirkland Lake." The author outlined the program of the investigations carried out since 1939 and gave some of the results already obtained. Equipment has been designed to pick up, amplify and record the small subaudible snaps which occur in a rock under pressure and which increase in number as the pressure increases. The records of a severe rock burst which occurred on January 29, 1943, show conclusively that the method clearly delimits the area under pressure to within a hundred feet or less. So far, attempts to predict bursts as to time have not been successful; but it is hoped that further work will result in some measure of time prediction.

Professor G. B. Reed, president of Section V (Biological Sciences), spoke on "Wound Infections and Local Chemotherapy." This paper dealt with the treatment of wounds with the sulfa drugs and the speaker emphasized the necessity of applying the drug as soon as possible after the wound has been inflicted in order to obtain the greatest benefit. Fifty-six papers were presented to this section, many of which will appear in scientific journals. Space permits the mention of the two invited papers only. The one by Dr. Babkin, on "Secretory Mechanism of the Digestive Glands," contained a survey of the work of his laboratory on this subject. Briefly it might be stated that the investigations established that the mucous, demilune and myo-epithelial cell groups of the submaxillary gland each have a separate innervation, and that the surface epithelium cells of the gastric mucosa and the mucoid, peptic and parietal cells of the gastric glands are under independent nervous or humoral control. The conclusions to be derived from this work were stated and further analysis of the secretory function of the digestive glands given. The second paper, by Professor G. W. Scarth, on "The Mechanism of Frost Resistance," contained an account of the modes of frost injury to plant cells and of the protoplasmic changes which accompany frost-hardening. He also discussed how the different hardening changes afford protection. The new president of this section is Professor H. S. Jackson.

At the general meeting of the society, Monsignor Olivier Maurault, rector of the University of Montreal and a fellow of Section I, was elected president of the Royal Society of Canada and Professor J. K. Robertson was elected vice-president.

DAVID A. KEYS

SPECIAL ARTICLES

THE PRODUCTION OF FOLIC ACID BY RAT LIVER IN VITRO

A PRELIMINARY study,[1] using *Streptococcus lactis R*, indicated that the urine of man contains only very small amounts of folic acid. We have found, using *Lactobacillus casei*, that the daily urinary excretion of this factor by 15 normal individuals, based on the assay of 42 samples, averages 0.0108 mgm units (0.0038 to 0.0238 mgm units).[2] The average daily folic acid intake of well-fed adults has been reported to be about 1.4 mgm units per day.[3] Evidently folic acid is unlike other members of the vitamin B complex (thiamine, riboflavin, pantothenic acid, biotin) in that only a trace appears in the urine of man (< 1 per cent. of the probable dietary intake).

To test the possibility that the folic acid is excreted in the urine in a complex form without microbiological activity, urine samples were subjected to autoclaving, autoclaving with very dilute acid or alkali, and digestion with takadiastase (an enzyme preparation used to release folic acid from tissue combination[2]). These procedures did not increase the amount of folic acid found in the urine. We have observed, however, that incubation of urine with a fresh rat liver preparation causes the appearance of more folic acid than can be accounted for by the analysis of the constituents of the digestion mixture. In conducting such experi-

[1] L. D. Wright, J. R. McMahan, V. H. Cheldelin, A. Taylor, E. E. Snell and R. J. Williams, University of Texas Publication, 4137: 38, 1941.

[2] V. H. Cheldelin, M. A. Eppright, E. E. Snell, B. M. Guirard, University of Texas Publication, 4237: 15, 1942.

[3] R. J. Williams, *Jour. Am. Med. Asn.*, 119: 1, 1942.

ments we have usually employed 10 cc of urine, or of other test materials dissolved in water, to which has been added 10 cc of a 20 per cent. suspension of fresh rat liver (2 parts of liver ground in a Waring blendor with 8 parts of M/10 phosphate buffer, pH 7). In some experiments we have used a weighed sample of liver fragmented in phosphate buffer with a spatula. Takadiastase (0.4 per cent.) is added, but this does not participate in the formation of folic acid by the liver preparation, under the influence of added substances. The mixtures are covered with a thin layer of benzene and allowed to incubate at 37° C. for 18 to 24 hours. After incubation folic acid is determined in the tissue filtrates by microbiological methods.[4]

Using the technics described we have investigated the material present in urine and the conditions requisite to the formation of folic acid by the rat liver preparation. No increase in the folic acid content occurs, over that calculated from the analyses of the constituents, if a heated rat liver preparation is used. The reaction is inhibited by high concentrations of NaCl and by M/500 NaCN and does not occur if liver extracts are used instead of fresh rat liver. The material in urine is stable to autoclaving for 1½ hours in the presence of normal HCl or H_2SO_4, under which circumstances folic acid itself is destroyed. The substance is readily absorbed on fullers' earth[5] at pH 3 and may be eluted with ammoniacal 50 per cent. alcohol. Using such procedures the material in urine has been concentrated approximately 2,000 times. When other natural materials (grass and liver extracts) are autoclaved with acid and then incubated with liver tissue, more folic acid is usually found on assay of the filtrates than can be accounted for by the analyses of the materials tested.

The stability to acid and the adsorption-elution characteristics of the substance in urine suggest that it may be related to uropterin (a urinary pigment),[6] or xanthopterin (the pigment of yellow butterfly wings),[7] which is claimed to be identical with uropterin.[6] When synthetic xanthopterin[8] is incubated with liver tissue a significant increase in the folic acid content of the filtrate from the mixture is usually found. This increase may be demonstrated with either *L. casei* or *Strep. lactis*. Before incubation with rat liver xanthopterin is entirely ineffective in replacing folic acid as a growth factor for either organism. Xanthopterin alone, among the pure compounds thus far tested, causes the formation of an increased amount of folic acid when incubated with rat liver. The following substances have been found ineffective: adenine, guanine, xanthine, uracil, cytosine and synthetic leucopterin[8] (the pigment of white butterfly wings[7]).

Preliminary evidence indicates that under certain circumstances the yield of folic acid, obtained by incubating liver with superfiltrol-eluates from acid-autoclaved liver extracts, may be materially increased by the addition to such mixtures of an aqueous extract of an acid ether extract of acid-autoclaved liver. Such an extract contains a considerable amount of acidic organic material, but is, of course, devoid of folic acid. An attempt to identify the substance or substances responsible for the effect of this material is now in progress. It is possible that the yields of folic acid obtained, when the xanthopterin-like material in acid-autoclaved liver extracts is incubated with rat liver, are limited by the supply of substances related to those found in the ether soluble fraction.

Discussion

Our experiments indicate that a substance stable to both heat and normal acid occurs in either free or combined form in urine, grass and liver. It appears likely that in some materials (grass) a considerable portion of this substance is derived from the degradation of folic acid, in other materials (urine and some liver extracts) the amount formed by autoclaving with acid is too large to have been derived from folic acid alone. The substance, when incubated with a preparation of fresh rat liver, causes the formation of folic acid; an effect also produced by synthetic xanthopterin. The substance obtained from urine, grass and liver has not yet been identified; however, the evidence suggests that it is related to xanthopterin.

Mitchell[9] has claimed that xanthopterin occurs in folic acid concentrates, and Totter and Day[10] have reported that xanthopterin, like concentrates of folic acid, promotes growth and cures leucopenia in rats fed succinylsulfathiazole in highly purified rations. Although neither we nor others[11] have been able to confirm this finding with xanthopterin, we believe a possible explanation of the conflicting data may be based on our results. A personal communication from Dr. Day suggests that age or duration of feeding the purified diet might be involved. It is possible that the response obtained from xanthopterin in rats may be limited by the availability of another substance. Such a possibility is suggested by the results we have obtained with material extracted by ether from a liver

[4] M. Landy and D. M. Dicken, *Jour. Lab. and Clin. Med.*, 27: 1086, 1942.

[5] Superfiltrol (thiamine grade).

[6] W. Koschara, *Zeit. f. physiol. Chem.*, 240: 127, 1936.

[7] C. Schöpf and E. Becker, *Ann.*, 507: 266, 1933.

[8] The syntheses of xanthopterin and leucopterin were generously carried out by our colleagues, Drs. A. M. Land and J. M. Sprague, according to the methods of R. Purrmann, *Ann.*, 544: 182, 1940 and 546: 98, 1940.

[9] H. K. Mitchell, SCIENCE, 97: 442, 1943.

[10] J. R. Totter and P. L. Day, *Jour. Biol. Chem.*, 147: 257, 1943.

[11] Personal communication from several laboratories.

TABLE 1

THE FOLIC ACID CONTENT OF RAT LIVER AFTER INCUBATION WITH VARIOUS SUPPLEMENTS

Expt. No.	Incubation mixture (Liver plus Supplement)	Folic Acid Content per gram of		Folic Acid formed per gram of liver
		Liver alone	Liver plus Supplement	
		μ gm units*	μ gm units*	μ gm units*
1	Liver (2.0 gm) + Urine (10 cc = 0.08 μ gm units of folic acid)	9.2	17.2	8.0
2	Liver (2.0 gm) + Urine (10 cc = 0.08 μ gm units of folic acid)	16.0	26.0	10.0
3	Liver (4.8 gm) + Urine (20 cc = 0.16 μ gm units of folic acid)	10.0	30.8	20.8
4	Liver (3.0 gm) + Urine (10 cc)	6.8	12.8	6.0
	Liver (3.0 gm) + Urine (10 cc) after treatment with superfiltrol	6.8	8.8	2.0
	Liver (3.6 gm) + Superfiltrol eluate ≅ 10 cc urine	6.8	12.9	6.1
5	Liver (2.5 gm) + Urine (10 cc = 0.13 μ gm units of folic acid)	10.4	14.4	4.0
	Liver (2.1 gm) + Acid-autoclaved urine (10 cc = 0.00 μ gm units of folic acid)	10.4	23.2	12.8
6	Liver (2.0 gm) + Xanthopterin (250 μ gm)	8.0	14.0	6.0
7	Liver (2.0 gm) + Xanthopterin (250 μ gm)	5.6	17.2	11.6
8	Liver (2.0 gm) + Xanthopterin (250 μ gm)	4.0	10.4	6.4
9	Liver (2.0 gm) + Adenine, guanine, xanthine, uracil or cytosine (250 μ gm)	4.4–4.8	4.4–4.8	0.0
	Liver (2.0 gm) + Xanthopterin (250 μ gm)	4.6	15.2	10.6
10	Liver (2.0 gm) + Leucopterin (250 μ gm)	16.0	14.4	0.0
11	Liver (2.9 gm) + Armour's parenteral liver extract (15 anti-P.A. units per cc) (0.1 cc = 1.4 μ gm units of folic acid)	20.0	26.0	6.0
12	Liver (2.6 gm) + Powdered Liver Concentrate (Wilson's 1 : 20), 200 mgm = 3.0 μ gm units of folic acid per gram of rat liver present	8.4	11.2	2.8
	Liver (3.1 gm) + Acid-autoclaved Powdered Liver Concentrate (Wilson's 1 : 20), 200 mgm = 0.02 μ gm units of folic acid per gram of rat liver present	8.4	36.4	28.0

* Results are expressed in terms of μ gm "potency 40,000 material," as described by V. H. Cheldelin, *et al.* (footnote 2).

extract autoclaved with acid. Such a substance might be stored to some extent in the liver of rats on normal diets, and might be derived from either dietary sources or from bacterial synthesis in the intestine. With continued feeding of purified diets or of purified diets containing succinylsulfathiazole, liver storage would be progressively reduced and the influence of xanthopterin on the synthesis of adequate amounts of folic acid in the liver of the rat would be inhibited.

Xanthopterin has been reported as effective in the cure of goats' milk anemia in rats[12] and as an anti-anemia factor for fish.[13] Evidence has recently been presented which indicates the identity of folic acid with a crystalline chick anti-anemia factor (vitamin B_c),[14] which has enormous activity as a growth factor for *L. casei* in the absence of folic acid concentrates,[14] and which functions similarly for *Strep. lactis.* Concentrates containing folic acid have proved effective in the treatment of leucopenia and anemia in the rat on purified diets containing poorly absorbed sulfonamides,[15, 16] and in the monkey on purified diets alone.[17, 18] In one monkey alleviation of leucopenia and anemia followed the use of xanthopterin when a liver powder prepared by heating fresh liver at 100° C. for 24 hours was also fed. Neither substance was effective by itself.[19]

It appears reasonable to suggest that from xanthopterin or closely related compounds may be derived substances concerned with the formation of erythrocytes and granulocytes. Various animal species appear to vary in the complexity of their requirements for hemocytopoietic material. In man the utilization of dietary anti-anemia material (extrinsic factor) is known to require the participation of an additional substance found in gastric juice (intrinsic factor), for the formation of what appears to be a more complex

[12] R. Tschesche and H. J. Wolf, *Zeit. f. physiol. Chem.*, 248: 34, 1937.

[13] R. W. Simmons and E. R. Norris, *Jour. Biol. Chem.*, 140: 679, 1941.

[14] J. J. Pfiffner, S. B. Binkley, E. S. Bloom, R. A. Brown, O. D. Bird, A. D. Emmett, A. G. Hogan and B. L. O'Dell, SCIENCE, 97: 404, 1943.

[15] S. S. Spicer, F. S. Daft, W. H. Sebrell and L. L. Ashburn, *Pub. Health Rep.*, 57: 1559, 1942.

[16] A. E. Axelrod, P. Gross, M. D. Bosse and K. F. Swingle, *Jour. Biol. Chem.*, 148: 721, 1943.

[17] W. C. Langston, W. J. Darby, C. F. Shukers and P. L. Day, *Jour. Exper. Med.*, 68: 923, 1938.

[18] S. Saslaw, H. E. Wilson, C. A. Doan and J. L. Schwab, SCIENCE, 97: 514, 1943.

[19] J. R. Totter, C. F. Shukers, J. Kolson, V. Mims and P. L. Day, *Fed. Proc.*, 2: 72, 1943.

substance (anti-pernicious anemia factor). Assays of several liver extracts intended for parenteral use in pernicious anemia therapy have shown the presence of small amounts of folic acid, and of larger amounts of the substance(s) formed by autoclaving with acid.

SUMMARY

The occurrence in urine and in acid-autoclaved grass and liver extracts of a substance which appears to participate in the synthesis of folic acid by rat liver *in vitro* is described. A similar effect is produced by synthetic xanthopterin. The effect of these materials might be accomplished by (1) catalysis of the enzymatic synthesis of folic acid; (2) the release of folic acid not liberated by takadiastase from tissue complexes; or (3) their serving as substrate material for the enzymatic synthesis of folic acid. The data presented favor the last hypothesis and suggest that xanthopterin, or a substance derived from it, may constitute a portion of the folic acid molecule. The probable involvement of compounds related to xanthopterin in the formation of hemocytopoietic substances in several animal species is discussed.[20]

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DISTRIBUTION AND HEREDITY OF VARIANTS OF THE RH TYPE[1]

WITH the aid of anti-rhesus immune sera, Landsteiner and Wiener[2] divided human beings into two classes, Rh positive and Rh negative, the former comprising about 84 per cent. of white individuals in New York City. These authors[3] also showed that the Rh factor is inherited as a simple mendelian dominant, independently of the blood groups and M-N types. The Rh factor has proved to be of considerable clinical importance in the etiology of intragroup hemolytic transfusion reactions[4,5] and in the pathogenesis of erythroblastosis fetalis.[6,7,8]

It was early recognized that the Rh factor is antigenically and genetically complex. Thus, Wiener[5] described an anti-Rh serum which agglutinated only about 70 per cent. of all bloods from white individuals, and pointed out that with the aid of this special anti-Rh serum the Rh type can be subdivided into two subtypes analogous to the major subgroups of A. According to the terminology suggested by Wiener and Landsteiner,[9] Rh positive bloods reacting with the special anti-Rh serum (now designated anti-Rh_1) belong to type Rh_1, while the remainder (about one sixth of the Rh positive bloods) belong to type Rh_2. As Wiener and Landsteiner[9] have shown, the types Rh_1, Rh_2 and negative are inherited by means of triple allelic genes Rh_1, Rh_2 and rh, where Rh_1 and Rh_2 are both dominant over rh and and Rh_1 is dominant over Rh_2.

The situation is further complicated by the fact that some bloods of subtype Rh_1 (reacting with anti-Rh_1 serum) do not react with guinea-pig anti-rhesus serum, so that a fourth type is determined in that way.[3] This type (designated as Rh′) is rare, comprising only about 3 per cent. of all white individuals. It should be mentioned that to allow for this special type, it was originally suggested that the standard guinea-pig anti-rhesus (giving about 84 per cent. positive reactions) be designated as anti-Rh_1, and the anti-Rh serum giving 70 per cent. positive reactions as anti-Rh_2.[10] This terminology has been abandoned, however, because it was found that the hypothetical agglutinogens determined in this way do not mendelize like A and B but are apparently "partial antigens" like the factors B_I, B_{II}, B_{III}, . . . of human group B blood, and the factors F_A, A_1, A of group A_1 blood.[11] In the present communication, only the major subtypes, Rh_1 and Rh_2, and not type Rh′, will be included in the discussion.

Recently, Wiener and Sonn[12] have described a serum from a mother of an erythroblastotic infant, which contained a potent anti-Rh agglutinin reacting

20 We are indebted to Miss Helen Ryan for capable assistance; to Dr. W. R. Graham, of the Cerophyl Laboratories, for generous supplies of grass juice powder; to Dr. David Klein, of the Wilson Laboratories, for the powdered liver concentrate (1 : 20). Some of the data in this paper were presented before the Philadelphia Physiological Society on May 18, 1943; an abstract appears in the *Am. Jour. Med. Sci.*, 206: 128, 1943.

1 From the Serological Laboratory of the Office of the Chief Medical Examiner of New York City. Aided by a grant from the Carnegie Foundation and the Committee on Human Heredity of the National Research Council.

2 K. Landsteiner and A. S. Wiener, *Proc. Soc. Exp. Biol. and Med.*, 43: 223, 1940.

3 K. Landsteiner and A. S. Wiener, *Jour. Exp. Med.*, 74: 309, 1941.

4 A. S. Wiener and H. R. Peters, *Ann. Int. Med.*, 13: 2306, 1940.

5 A. S. Wiener, *Arch. Path.*, 32: 227, 1941.

6 P. Levine, E. M. Katzin and L. Burnham, *Jour. Am. Med. Assoc.*, 116: 825, 1941.

7 P. Levine, L. Burnham, E. M. Katzin and P. Vogel, *Am. Jour. Obstet. and Gynec.*, 42: 925, 1941.

8 L. Burnham, *Am. Jour. Obstet. and Gynec.*, 42: 389, 1941.

9 A. S. Wiener and K. Landsteiner, *Proc. Soc. Exp. Biol. and Med.*, 53: 167, 1943.

10 As was first shown by Levine, and confirmed by Wiener, the most common human anti-Rh sera, now designated anti-Rh′, giving about 87 per cent. positive reactions, contain two agglutinins, one corresponding to the 84 per cent. sera (anti-Rh), the other to the 70 per cent. sera (anti-Rh_1).

11 A. S. Wiener, "Blood Groups and Transfusion," 3rd edition, p. 254. Springfield, Ill.: C. C Thomas. 1943.

12 A. S. Wiener and E. B. Sonn, *Jour. Immunol.*, in press.