greater than treatment B ($P < 0.01$). The results are presented in Fig. 1.

A single 2-g dose of Benemid (treatment B) elevates by 1.6 to 2.1 times the plasma concentrations of PAS observed 4, 6, and 8 hr after a single 4-g dose of PAS. A daily dose of 2 g of Benemid administered in 0.5-g doses every 6 hr (treatment C) elevates by 2.3 to 4.1 times the plasma concentrations of PAS observed at the same time intervals after 4 g of PAS.

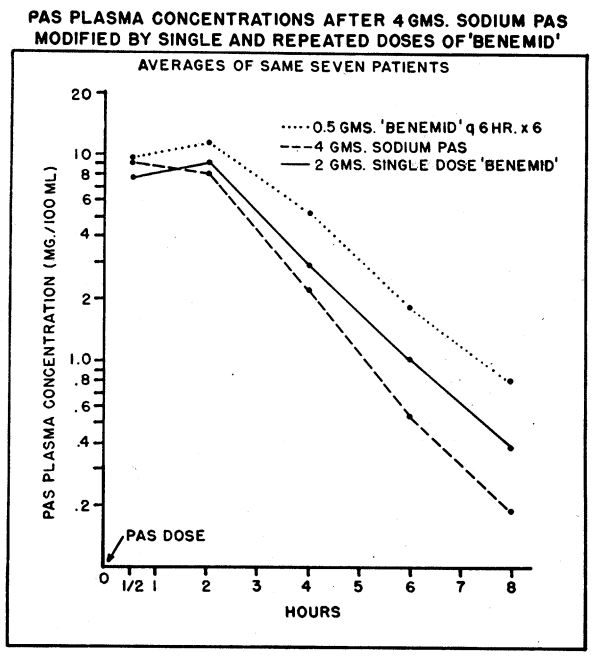

FIG. 1.

Because of the stability of Benemid and the absence of a free amine group in its structure, noninterference of the compound with the Way method might be anticipated. Repeated tests have shown that Benemid does not give a color reaction when samples were analyzed by the Way procedure.

Benemid, a crystalline white powder, is nearly insoluble in water.[5] Initially the drug is tasteless but a bitter taste is noted occasionally, which is then displaced by a pleasant aftertaste. The drug is absorbed rapidly from the gastrointestinal tract, and, after a single oral dose administered to dogs, it can be demonstrated in the plasma for as long as 36 hr. Nearly 75% of the drug is bound to plasma proteins and it is excreted in the urine almost entirely in conjugated form, probably as a glucuronide. Both acute and chronic toxicity studies in mice and dogs have shown a high therapeutic index for Benemid (*4*). Benemid has been administered daily to human patients for 3 weeks without any observed toxicity.

PAS is conjugated before excretion, and, of the total amount of PAS excreted in the urine, "approximately 59% is AcPASA, 18% is PASA, 13% is *p*-aminosalicyluric acid and the remainder represents one free amine and one conjugated amine which are highly water soluble" (*5*). It is suggested that Benemid inhibits the conjugation of PAS so that the drug is presented to the kidney for excretion in a form that is less rapidly excreted than are the conjugates of PAS. An adequate dose of Benemid in combination with PAS would be expected to result in more prolonged and higher plasma concentrations of PAS, and on the basis of the observations here reported, the plasma concentrations of PAS are enhanced two to four times. Therefore, Benemid may extend and greatly increase the efficacy of PAS in the treatment of tuberculosis.

[5] The chemistry of this substance and related substances will be published by Miller, Zeigler, and Sprague.

References

1. BEYER, K. H., PAINTER, R. H., and WIEBELHAUS, V. D. *Amer. J. Physiol.*, to be published.
2. BEYER, K. H. Personal communication.
3. ERDEI, A., and SNELL, W. E. *Lancet*, 1948, 791.
4. RAGAZ, L. Z. *Schweiz. med. Wschr. J. Suisse Med.*, 1948, **78**, 1213.
5. WAY, E. L., *et al. J. Pharm. exp. Therap.*, 1948, **93**, 368.

The Composition of Meconium: Isolation of Blood-Group-Specific Polysaccharides. Abnormal Composition of Meconium in Meconium Ileus

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Meconium, the first intestinal discharge of the newborn, differs in appearance and properties from the stools of later life. It represents material accumulated during fetal life and is free of bacteria or their breakdown products. In the human infant it is blackish-green, odorless, has a viscid, sticky consistency, and varies in amount from 60 to 200 g. It is first demonstrable during the fifth month of gestation. Generally it is considered to be an accumulation of debris consisting of desquamated cells of the alimentary tract and skin, lanugo hairs, fatty material from the vernix caseosa, amniotic fluid, and various intestinal secretions. Its color is thought to be due to bile pigments (*12*). In a pathologic condition called "meconium ileus" (*8*), the amount of meconium is greater and its consistency even more viscid than is normal. This disorder is thought to represent the earliest and most severe form of cystic fibrosis of the pancreas, a disease characterized by diminution or absence of pancreatic enzymes. Neither normal nor abnormal meconium has as yet been studied by modern methods.

Analysis of meconium. In Table 1 is listed the composition of a pooled sample of normal meconium, as well as that of a specimen obtained from an infant with meconium ileus. Considering first the normal meconium, it may be seen that, as compared with adult stools, the

TABLE 1

ANALYSIS OF MECONIUM OF NORMAL INFANTS AND PATIENT WITH MECONIUM ILEUS*

Meconium	Dry weight	On dry weight basis					Non-protein N	Purines	Free reducing sugar	Acid-soluble phosphorus
		Ash	Nitrogen	Alcohol-ether extractable	Total reducing sugar	Total phosphorus				
	%	%	%	%	%	%	% of total N	% of total N	% of total sugar	% of total P
Normal:	27.6	4.0	6.9	12.3	35.5	0.04	84.1	1.4	3.1	57
Ileus:	29.8	1.8	14.4	9.7	8.7	0.07	5.4	2.9	2.7	9

* The determinations for dry weight, ash, nitrogen, material soluble in alcohol-ether, and total phosphorus were performed according to standard methods. The total sugar was determined after hydrolysis for 2 hr at 100° C in normal acid by the method of Nelson (7). Nonprotein N and acid-soluble P were estimated on the trichloroacetic acid filtrate. The purines were determined according to Hitchings (4) with the use of double precipitation.

water content was high, and the ash content extremely low. Nitrogen, purine, and phosphorus, even on a dry-weight basis, were low and, for the most part, water- and trichloracetic acid soluble. No protein could be demonstrated by heat or the usual precipitants. Relatively little lipid material was present. The most prominent component appeared to be a polysaccharide, which yielded a reducing sugar after acid hydrolysis. These data are incompatible with the prevailing ideas about the composition of meconium as a chance collection of debris; they indicate the presence of a specific component, carbohydrate in nature, as a consistent major constituent.

The meconium from the patient with ileus differed markedly in composition from the normal. It was much higher in nitrogen, which for the most part was precipitable by trichloroacetic acid, as were the phosphorus and purine. Qualitative protein reactions were positive. The percentage of carbohydrate was relatively low. The absolute amount, however, may well have been normal; although a quantitative collection was impossible, it appeared that the amount of meconium was much greater than usual.

The next phase of the study was directed toward elucidation of the nature of the carbohydrate. It appeared to exist in combination with nitrogenous substances and to represent a mucopolysaccharide. The polysaccharide was water soluble and precipitable by 10 volumes of glacial acetic acid or 5 volumes of ethanol. It was not precipitated by various deproteinizing agents. Most of the bile pigment could be removed by acetic acid, alcohol, or treatment with barium hydroxide and $ZnSO_4$ (Somogyi deproteinization). The solutions of the polysaccharide were translucent, with a light yellow or greenish tinge. The reducing substance was nonfermentable. Specific color reactions were positive for glucosamine, galactose, and methylpentose. Mucic acid could be isolated. Hexuronic and sulfuric acids were absent. A product prepared by preliminary removal of interfering substances by barium hydroxide and $ZnSO_4$, followed by precipitation with glacial acetic acid, yielded the following data: N, 4.3%; reducing sugar (Somogyi-Nelson), 32.1%; glucosamine, 18.8%; acetyl, 6%. Further preparations were made following the procedures of Morgan and King (*6*) or Sevag (*11*). Their composition is discussed in a later section. The general similarity of the polysaccharide to blood group substances prompted the investigation of the blood group activity of meconium.

TABLE 2

BLOOD GROUP ACTIVITY OF BLOOD, SALIVA, AND MECONIUM*

Sample of meconium	Blood	Saliva	Approximate amount of meconium inhibiting isoagglutination	
			A	B
			µg	µg
All.	O	—	—	—
Wil.	O	—	—	—
Fel.	A	A	0.1	—
Kel.	A	A	0.1	—
McD.	A	A	0.1	—
Tho.	A	A	0.05	—
Wei.	A	A	0.7	—
Bon.	B	B	—	0.7
Hug.	B	—	—	—
Spr.	B	B	—	0.7
Wil.	AB	AB	60	60
Wri.	AB	AB	70	10

* The tests were carried out according to the technique of Morgan and King (*6*): 0.1 ml of diminishing concentrations of the material was mixed with 0.1 ml of human anti-A or anti-B serum containing 20–40 agglutinating doses. After standing 1 hr, 0.1 ml of an 0.5% suspension of standardized A or B erythrocytes in 0.9% NaCl was added. After 2 hr at room temperature, the mixtures were centrifuged and examined macro- and microscopically for agglutination. Minimal agglutination was taken as the end point. The figures in the table refer to the amount of material in 0.1 ml of solution tested. The dashes indicate lack of activity with the highest amount tested, usually 1,000 µg.

Blood group activity of meconium.[1] The activity of meconium was assessed by its ability to inhibit isoagglutination according to the procedure of Morgan and King (*6*), with 20–40 agglutinating doses of human anti-A or anti-B serum. It was found that a sample representing a pool of 25 meconium stools neutralized both A and

[1] Blood group activity of meconium has been noted by several observers (*9, 10, 13–15*).

TABLE 3

COMPOSITION OF TYPE-SPECIFIC POLYSACCHARIDES OF MECONIUM

Fraction	Nitrogen	Reducing sugar	Hexosamine*	Acetyl*	Methyl pentose*	Ratio	Ratio	Ratio	Blood group activity*	
									A	B
	%	%	%	%	%	4/2	4/3	5/4	μg	μg
Analysis of pooled meconium†										
Crude	7.1	26.2	15.3	5.0	3.8	.17	.59	1.36	2.5	30
Mo. I	5.4	34.7	23.2			.33	.67		10	25
Mo. II	5.1	43.9	28.4	8.9	6.0	.43	.65	1.30	0.5	25
Mo. III	5.8	22.4	13.5			.19	.60		2.5	250
Sevag III	6.0	42.6	25.9	9.6	5.3	.34	.61	1.54	0.1	16
Analysis of individual meconiums										
Sevag purification										
McD. Group A	6.6	37.3	23.2	8.2	7.7	.27	.62	1.47	0.01	—
Bon. Group B	6.7	37.0	22.0	6.6	6.0	.25	.60	1.24	—	0.01
Wil. Group O	6.7	40.0	23.0	7.5	5.2	.27	.58	1.36	100?	—
Wri. Group AB‡	6.2	41.6	24.8	7.4	8.4	.31	.60	1.24	—	0.2
Calf meconium										
Crude	6.2	12.0	6.8		1.0	.08	.57		—	—
Sevag	5.4	36.6	22.8	8.0	4.6	.33	.62	1.46	—	—

* Hexosamine was determined according to Elson and Morgan (*3*), acetyl, by a modified method of Kabat and Mayer (*5*), methyl pentose by the CyR10 reaction according to Dische (*2*), and the blood group activity by the procedure of Morgan and King (*6*).

† The fractionation was performed according to Morgan and King (*6*). Fraction Mo. I represents material insoluble in 90% phenol, fraction Mo. II that precipitating in 27% alcoholic phenol, and fraction Mo. III, the material insoluble in 50% alcoholic phenol. The Sevag purifications were performed by shaking repeatedly aqueous solutions of meconium brought to pH 4.8 by the addition of sodium acetate and acetic acid, with one-fourth volume of a 10 : 1 mixture of carbon tetrachloride and amyl alcohol. After four to eight extractions, the supernatant material was precipitated with six volumes of absolute alcohol.

‡ The blood cells of Wri. were only weakly agglutinated by anti-A, but strongly by anti-B serum. The crude meconium showed A group activity in amounts of 70 μg but B activity with 10 μg. On purification the A activity disappeared. Possibly it represented a subgroup of A.

B agglutinating sera in amounts of 25 μg and 300 μg, respectively. The next step was to determine whether the activity of the meconium of an individual infant's stool corresponded to his blood group and whether a distinction existed between "secretors," i.e., infants whose body secretions contain water-soluble, group-specific substances, on the one hand, and nonsecretors on the other, i.e., infants whose body fluids lack this property. Individual samples of meconium, blood, and saliva from a group of 12 infants were tested. It may be seen from Table 2 that the activity of meconium paralleled the properties of saliva. Both saliva and meconium of the infants of blood group O were devoid of A or B activity, whereas the materials from infants of blood group AB had both A and B activity. Meconium from infants of group A showed somewhat higher activity than that from infants of group B and both were considerably more potent than meconium from infants of group AB. Both saliva and meconium of one nonsecretor of blood group B were found inactive, indicating that the activity of meconium is restricted to secretors.

Chemical composition of polysaccharides of meconium. Table 3 summarizes the chemical data on polysaccharides obtained from pooled and individual meconiums from infants of different blood groups. It may be seen that, on the whole, the composition of the purified fractions is in agreement with products obtained from other sources (*1*). It is noteworthy that the crude meconium had a high polysaccharide content, estimated from its reducing value to represent over 80% of its weight, but that it contained an excess of nitrogenous substances. Fraction Mo. II, corresponding to the best fraction of Morgan and King (*6*), was highest in sugar and lowest in nitrogen content, and would appear to be purest by these standards. However, its blood group activity was, if anything, lower than that of the Sevag III fraction, which was higher in N and lower in sugar. There was evidence of fractionation in the phenol procedure between A and B activity of the pooled meconium, the B activity being associated with the most insoluble fraction, Mo. I. The Morgan procedure gave distinctly inferior yields of polysaccharide as compared to the Sevag method, which led to a recovery of about 50% of the reducing sugar of meconium in purified form. The analysis of polysaccharides of individual meconiums of different blood groups showed only minor differences in composition. Those from individual meconiums of A or B individuals were considerably more active than the preparation from pooled stools. They appeared to be even more potent than the products described by Morgan, but little stress should be laid on differences observed in different laboratories, in view of the nature of the serologic method. The analyses on the meconium from a calf fetus[2] indicated

[2] Obtained through the courtesy of Louis Kahn, of Cincinnati.

a polysaccharide content much lower than that of human meconium. The purified polysaccharide, on the other hand, resembled the human polysaccharides closely, but was devoid of human blood group A or B activity.

The observations reported are as yet of a preliminary nature and require elaboration in several directions. From the present data, it would appear that meconium in the first instance represents the residue of the mucous secretions of the entire alimentary tract, including saliva and gastric and intestinal juices. It is known that in secretors the secretions of many glands normally contain mucoproteins and exhibit blood group activity. The absence of protein from normal meconium may be attributed to the activity of proteolytic enzymes, foremost tryptase, which would digest the protein while leaving the polysaccharides intact. The different composition of the meconium from the infant with meconium ileus might then be due to the lack of proteolytic activity, which would lead to the persistence of protein. The much greater than normal viscosity of meconium of infants with ileus would find its explanation in the circumstance that mucoproteins are more viscous than mucopolysaccharides. The observations on calf meconium would indicate that the occurrence of polysaccharides in meconium is not restricted to man.

The availability of a potent and easily accessible source of human blood group substances in the form of meconium appears of great theoretical and practical interest. It opens the possibility of study of hitherto practically inaccessible blood group substances and of a close comparison between human and animal products. Blood group substances from meconium offer great promise in blood transfusion practice as neutralizing agents of agglutinins of pooled plasma or blood, and have the advantages over currently used products of high potency, simple purification, and certain absence of antigenic properties.

References

1. BRAY, H. G. and STACEY, H. *Advances in carbohydrate chemistry.* New York: Academic Press, 1949. Vol. IV, p. 37.
2. DISCHE, Z., and SHETTLES, L. B. *J. biol. Chem.,* 1948, **175,** 595.
3. ELSON, L. A. and MORGAN, W. T. J. *Biochem. J.,* 1933, **27,** 1824.
4. HITCHINGS, G. H. *J. biol. Chem.,* 1941, **139,** 843.
5. KABAT, E. A. and MAYER, M. M. *Experimental immunochemistry.* Springfield, Illinois: Thomas, 1948.
6. MORGAN, W. T. J. and KING, H. K. *Biochem. J.,* 1943, **37,** 640.
7. NELSON, N. *J. biol. Chem.,* 1944, **153,** 375.
8. NELSON, W. N. (Ed.) *Mitchell-Nelson textbook of pediatrics.* Philadelphia and London: Saunders, 1945.
9. OKAJIMA, S. *Jap. J. med. Sci. VII, Soc. Med. and Hyg.,* 1940, **3,** 154.
10. SEKIMOTO, H. *Jap. J. med. Sci. VII, Soc. Med. and Hyg.,* 1940, **3,** 122.
11. SEVAG, M. G. *Biochem. Z.,* 1934, **273,** 419.
12. SMITH, C. A. *The physiology of the newborn.* Springfield, Illinois. Thomas, 1945.
13. UBEYAMA, R. *Jap. J. med. Sci. VII, Soc. Med. and Hyg.,* 1940, **3,** 139.
14. WITEBSKY, E. and SATOH, T. *Klin. Wschr.,* 1933, **12,** 948.
15. YOSIDA, K. I. *Z. ges. exp. Med.,* 1928, **63,** 331.

Taste Reactions to Antithyroid Substances

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Fox discovered a number of years ago (*4*) that some persons taste phenylthiourea (phenylthiocarbamide, or PTC) as distinctly bitter, whereas others find it nearly or quite tasteless. Family studies have shown (*2, 6*) that this taste ability is hereditary, the nontasting characteristic being in all probability a recessive gene. About 25% of most populations are nontasters for PTC, although there are racial and sexual variations. The discovery by Fisher, Ford, and Huxley (*3*) that chimpanzees can likewise be divided into tasters and nontasters for PTC makes it seem probable that this gene pair has existed in man for a very long time.

Thus, a gene appears to exist which enables its possessor to taste a synthetic compound not known to occur in nature. It is not too easy to understand how such a gene can exist, nor to guess what its function can be.

A possible explanation for this gene's existence appears to have been demonstrated in this laboratory. The substance *l*-5-vinyl-2-thio-oxazolidone, recently isolated and structurally defined (*1*), occurs widely in nature, particularly in turnips and cabbage. A sample was kindly sent by Dr. M. G. Ettlinger, and tests were made on 21 individuals, of whom 7 could not taste PTC, 13 tasted it as bitter, and 1 tasted it as bitter after some delay. Ability to taste *l*-5-vinyl-2-thio-oxazolidone was found to parallel exactly that for PTC. There can be little doubt that the same gene controls ability to taste this naturally occurring substance.

In regard to the "purpose" of the tasting gene, it is known that thiourea, thiouracil (*5*), *l*-5-vinyl-2-thio-oxazolidone, and other substances of similar constitution act as antithyroid drugs. This seems to point to some connection between the tasting gene and thyroid function. It is planned to test hypothyroid and hyperthyroid patients for ability to taste substances of this group and thus investigate further the possible relation between the "tasting" gene and glandular function.

It is realized, of course, that the relationship may be less direct than seems obvious at first. In fact, Fisher, Ford, and Huxley (*3*) suggest that the reason for the long survival in man of both the tasting genes might be that the heterozygote had some (unspecified) advantage over both the homozygotes. Examples of this have been observed in *Drosophila.*

References

1. ASTWOOD, E. B., GREER, M. A., and ETTLINGER, M. G. *J. biol. Chem.,* 1949, **181,** 121.
2. BLAKESLEE, A. F. *Proc. Nat. Acad. Sci.,* 1932, **18,** 120.
3. FISHER, R. A., FORD, E. B., and HUXLEY, J. S. *Nature,* Lond., 1939, **144,** 750.
4. FOX, A. L. *Proc. Nat. Acad. Sci.,* 1932, **18,** 115.
5. RIDDELL, W. J. B., and WYBAR, K. C. *Nature,* Lond., 1944, **154,** 669.
6. SNYDER, L. H. *Ohio J. Sci.,* 1932, **32,** 436.