erythrocyte counts per 10,000 red blood cells is presented. Routine counts within 24 hours of delivery are recommended on newly-born infants and more than 2 nucleated red blood cells per 10,000 are to be considered abnormal. Regardless of Rh compatibility the mortality among children with 5 or more nucleated cells per 10,000 was 67% in our small series, although among the Rh incompatible infants there were more nucleated red blood cells than among the Rh compatible group. The possibility of ill effects when the mother is Rh-positive and the infant Rh-negative is suggested by the data.

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A Microbiological Method for the Determination of Tryptophane.

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The widespread use of bacteria for the assay of the B-complex vitamins has drawn attention to their requirements for amino acids and their possible use as a basis of measurement of these substances. Detailed studies have been made of the amino acids required by *Lactobacillus casei*[1] and *Lactobacillus arabinosus* 17-5.[2] Bacterial methods of assay for the amino acids, especially tryptophane, have been under investigation in this laboratory and during the progress of this work there have appeared publications from other laboratories in which procedures have been outlined[3,4] for the use of *L. arabinosus* 17-5 in the assay of cystine, iso-leucine, methionine, leucine, valine, glutamic acid, threonine, and tryptophane. It seems desirable at this time to present the results of studies on the specificity of *l*-tryptophane for *L. arabinosus* 17-5 and methods for the preparation of proteins for assay. The importance of such studies is apparent since Snell[5] has recently found that indole or anthranilic acid may replace tryptophane for *L. arabinosus* 17-5, a fact which has also been observed in this laboratory.

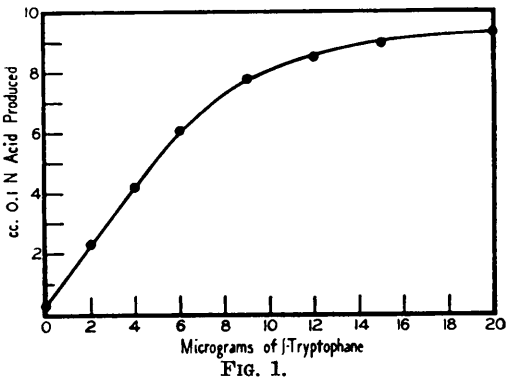

FIG. 1.

Although both *L. casei* and *L. arabinosus* 17-5 require tryptophane, the latter organism was selected for further study because its requirements appeared to be simpler. The Snell and Wright[6] niacin assay provided a technic readily adaptable to the assay of tryptophane. The basal medium differed only in the omission of tryptophane and the inclusion of niacin at a level of two micrograms per assay tube. The preparation and suitable combination of supplements have been described elsewhere.[6,7]

Specificity of Response to Tryptophane. The response of *L. arabinosus* 17-5 to pure *l*-tryptophane (Merck) is shown by a standard curve (Fig. 1) based on about 50 inde-

1 Hutchings, B. L., and Peterson, W. H., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 36.

2 Shankman, S., *J. Biol. Chem.*, 1943, **150**, 305.

3 Kuiken, K. A., Norman, W. H., Lyman, C. M., and Hale, Fred, *Science*, 1943, **98**, 266.

4 Shankman, S., Dunn, Max S., and Rubin, L. B., *J. Biol. Chem.*, 1943, **150**, 477.

5 Snell, E. E., *Arch. Biochem.*, 1943, **2**, 389.

6 Snell, E. E., and Wright, L. D., *J. Biol. Chem.*, 1941, **139**, 675.

7 Greene, R. D., Black, A., and Howland, F. O., *Ind. and Eng. Chem., Anal. Ed.*, 1943, **15**, 77.

TABLE I.
Activity of Pure Substances* for *L. arabinosus* 17-5 in a Tryptophane-free Medium.

Substance	γ per assay tube	% activity (molecular)
l-Tryptophane		100
dl-Tryptophane	5 to 20	48-50
Indole	2 to 8	60-90
Skatole	100	0
Indole-3-acetic acid	100	0
β(Indole-3)-propionic acid	100	0
γ(Indole-3)-n-butyric acid	100	0
Tryptamine hydrochloride	100	0
Anthranilic acid	2 to 8	30-50
Kynurenic acid	100	0

* Tryptophane (*l* and *dl*) was obtained from Merck and Co.; kynurenic acid was a Hofmann LaRoche product obtained through the courtesy of Dr. C. P. Berg, State University of Iowa, Iowa City; the other compounds were obtained from Eastman Kodak Co.

TABLE II.
Apparent Recovery of Tryptophane from Casein* under Various Conditions.

Condition of hydrolysis	Additions to hydrolyzed casein	Apparent % of *l*-tryptophane
5% pancreatin, 24 hr		1.00
" " " "	0.6% indole	1.47
" " " "	0.6% indole, followed by ether extraction	0.99
" " " "	0.6% anthranilic acid	1.33
" " " "	0.6% anthranilic acid, followed by ether extraction	1.02
5N $Ba(OH)_2$, 4 hr		0.54
" " 7 "		0.52
" " 10 "		0.51

* Smaco vitamin-free casein containing 8.0% moisture. On a theoretical basis of 15.6% N for pure casein the value for tryptophane would be 14.7% higher.

pendent series. Table I contains data on the activity of a number of substances which are related to tryptophane. A comparison of the activities of the *l*- and *dl*-forms indicates that *d*-tryptophane is not utilized by *L. arabinosus* 17-5. Indole and anthranilic acid were found to be highly effective in replacing *l*-tryptophane whereas various 3-substituted derivatives of indole were entirely inactive. Lugg[8] and Shaw and McFarlane[9] have discussed the possible interfering effect of indole in chemical tests and it thus appears necessary to exclude indole and anthranilic acid during microbiological assays for tryptophane. It has been found possible to separate both compounds from tryptophane by ether extraction at a pH of 4.0 (Table II).

Preparation of Materials for Assay. For the liberation of tryptophane from proteins pancreatic digestion and treatment with sodium or barium hydroxides were investigated. In a study of the digestion of casein with pancreatin, a procedure about the same as the U.S.P. method[10] was employed. A 0.1% solution of casein at pH 8.2 was digested at 37-38°C with pancreatin (Pfanstiehl 1/110) equal to 5% of the weight of the casein. Under these conditions the tryptophane values as followed by microbiological assay reached apparent equilibrium after about 20 hours. After adjustment to a pH of 4.0 with HCl and two extractions with an equal volume of ether the hydrolysate was brought to a pH of 6.8 for assay. Blank tryptophane values for the pancreatin were obtained by its autolysis and assay under the same conditions.

Tryptophane has been considered relatively stable to barium hydroxide[9,11] though subject to racemization. Although prelim-

[8] Lugg, J. W. H., *Biochem. J.*, 1938, **32**, 775.

[9] Shaw, J. L. D., and McFarlane, W. D., *Can. J. Res.*, 1938, **16B**, 361.

[10] U. S. P., 1936, XI, 276.

inary trials of sodium and barium hydroxide digestion of casein gave no striking differences, the latter seemed preferable and was investigated further. It seemed possible that hydrolysis might be conducted under conditions allowing complete racemization of tryptophane without appreciable destruction. A study of the effect of time and concentration of alkali indicated that hydrolysis of casein with 20 volumes of 5 N $Ba(OH)_2$ for about 5 hours in an autoclave at 15 lb pressure was a suitable treatment. After removal of Ba as $BaSO_4$ at a pH of 4.0 and extraction with ether the hydrolysate was prepared for assay by adjustment to a pH of 6.8 and a concentration of casein of 5 mg/cc. Assays of such hydrolysates against *l*-tryptophane standards yielded recovery values about one-half those obtained from pancreatic hydrolysates of casein and on the assumption of complete racemization of tryptophane during alkaline hydrolysis indicated close agreement between the two methods (Table II). It had been found earlier that pure *l*-tryptophane yielded a value close to 50% when assayed after a similar treatment with baryta.

This microbiological assay procedure for tryptophane appears to compare favorably in sensitivity and reproducibility with similar methods employed for the assay of the B-complex vitamins. A report of its application to a variety of proteins and foods will appear in a later publication.

Summary. Evidence was obtained which indicates that *L. arabinosus* 17-5 may be used for the assay of *l*-tryptophane. Of other related substances tested only anthranilic acid and indole produced a growth response and these are removed by ether extraction.

11 Gilman, H., *Organic Chemistry*, II, 1159, 2d Ed.

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Studies on the Absorption of Penicillin from the Stomach.*

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In a previous study,[1] penicillin was shown to be absorbed poorly when it was administered by mouth. In view of the fact that penicillin must be injected parenterally at such frequent intervals in the treatment of certain infections caused by gram-positive organisms, it seemed desirable to make further observations on the absorption of this antibacterial agent from the gastro-intestinal tract.

Methods. The sodium salt of penicillin† was dissolved in 0.85% sodium chloride in a concentration of 1,000 Oxford units per cubic centimeter. This standard solution was used in all experiments.

When penicillin was administered by mouth, 20,000 Oxford units were given in 200 cc of tap water. Frequent samples of blood were withdrawn from the anticubital vein and the serum then separated by centrifugalization. All urine passed for a period of 24 hours was stored as individual specimens at 5°C. The concentration of penicillin in these samples was determined by a method described previously.[2] Gastric juice, bile, and succus entericus were obtained by a Miller-Abbott tube; saliva was collected in a sterile container. When these substances were tested for their destructive effect upon penicillin, strains

* Supported by a grant from the Johnson Research Foundation, New Brunswick, New Jersey.

† Penicillin used in this study was supplied through the courtesy of Dr. George A. Harrop, Squibb Institute for Medical Research, New Brunswick, New Jersey.

1 Rammelkamp, C. H., and Keefer, C. S., *J. Clin. Invest.*, 1943, **22**, 425.

2 Rammelkamp, C. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 95.