VAPOUR-LIQUID EQUILIBRIUM DATA OF BENZENE – n – HEPTANE – n – BUTANOL SYSTEM

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ABSTRACT

Vapour-liquid equilibrium data of benzene-n-heptane-n-butanol system have been reported. The thermodynamic consistency of the system was tested with Li-Lu test and the data were correlated with Wohl's three suffix Margules equation.

Vapour-liquid equilibrium data of this system are not available in the literature. The system has been studied to find the effect of a third component *i.e.*, benzene on the binary azeotrope *i.e.*, n-heptane-n-butanol. The system has been studied under isobaric condition at 685 mm of Hg pressure.

EXPERIMENTAL

The equilibrium still and the experimental procedure have been described earlier[1]. The properties of the reagents used along with the literature values are given in Table I. Samples were analysed by the methods of refractive index and specific gravity. The compositions of mixtures are taken from the plot of composition versus physical properties.

TABLE I

Properties of pure components

Compound	Density		Refractive Index	
	Experimental	Literature[3]	Experimental	Literature[3]
Benzene	$0.87368^{25°C}$	$0.87369^{25°C}$	$1.5011^{20°C}$	$1.5011^{20°C}$
n-Heptane	$0.68380^{20°C}$	$0.68376^{20°C}$	$1.3880^{20°C}$	$1.3876^{20°C}$
n-Butanol	$0.80986^{20°C}$	$0.80978^{20°C}$	$1.3988^{20°C}$	$1.3991^{20°C}$

Thermodynamic Consistency

The vapour-liquid equilibrium data are presented in Table II, and the $x-y$ diagram is given in Fig. I. The liquid phase activity coefficients are calculated as,

$$\gamma_i = \frac{y_i \pi}{x_i P_i}$$

The vapour pressure data of the pure components at various temperatures are calculated using the equations given in the literature[3]. The data are correlated with Wohl's three suffix Margules equation[2]. It has been observed that the agreement of the experimental data with the calculated is better when the data are correlated using a ternary constant than when the constant was assumed to be zero. The value of constant has been obtained using experimental results and is found to be -0.306. The values of binary constants are obtained from the corresponding binary systems. The binary constants are:

$A_{12} = 0.087$ $A_{21} = 0.200$

$A_{23} = 0.800$ $A_{32} = 0.950$

$A_{31} = 0.380$ $A_{13} = 0\ 550$

The actual experimental values and the calculated values of vapour composition using Wohl's three suffix Margules equation, are also presented in Table II and are found to be in good agreement within reasonable limits of experimental error over most of the ranges of composition.

The thermodynamic consistency of the data was tested using Li and Lu method[4] and the data are found to satisfy the test. No ternary azeotrope exists in the range studied.

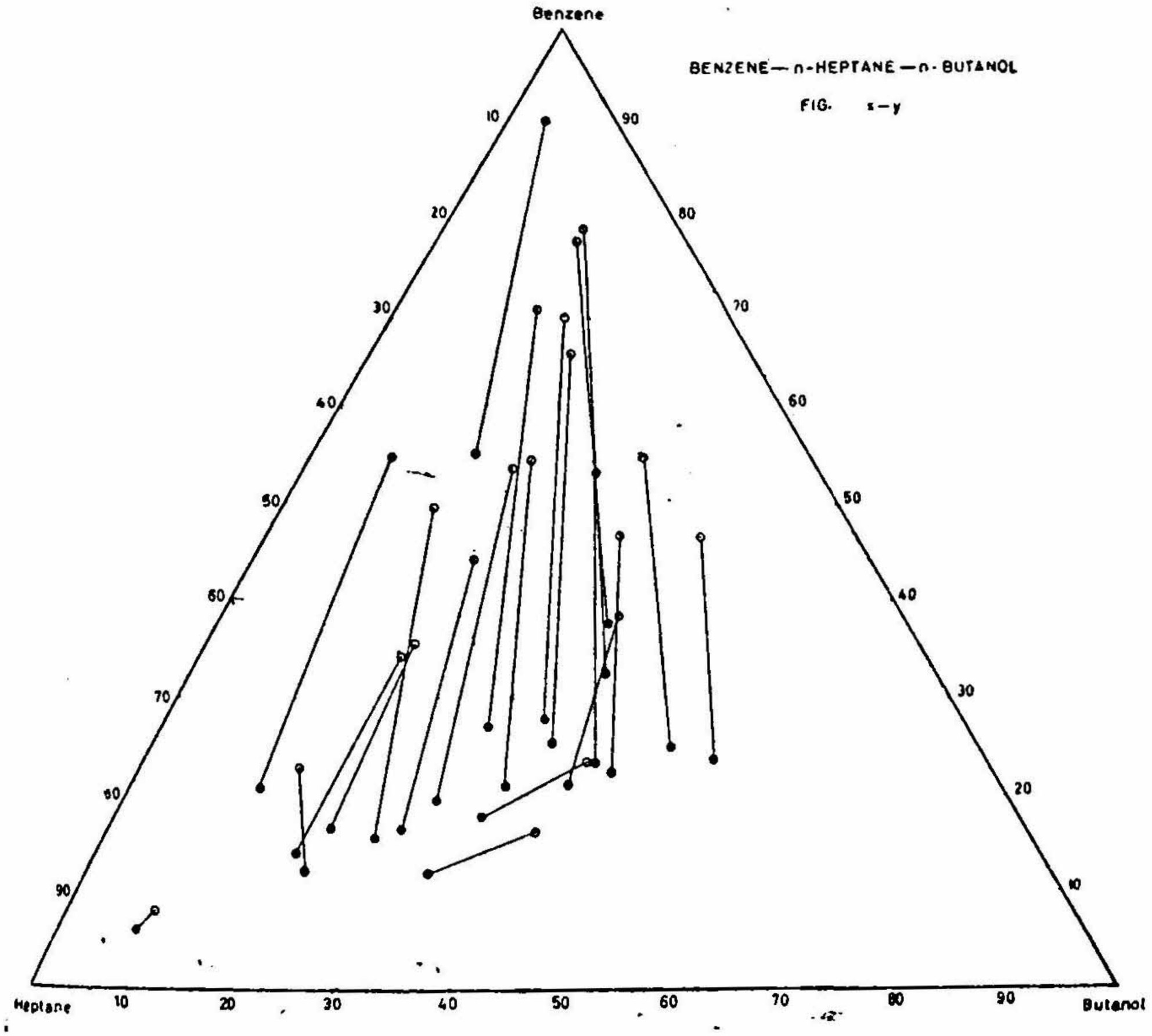
Nomenclature

P	.....	Vapour pressure of pure component
x	.....	Mole fraction in liquid phase
y	.....	Mole fraction in vapour phase
γ	.....	Activity coefficient
π	.....	Total pressure

TABLE II

System : Benzene (1) —n —Heptane (2) —n —Butanol (3)

No.	Temp. °C	x_1	x_2	x_3	y_1	y_2	y_3	γ_1	γ_2	γ_3	y_{1cal}	y_{2cal}	y_{3cal}
1	83.1	26.0	36.0	38.0	39.1	40.0	20.9	1.2328	1.5690	1.9157	38.47	41.50	19 25
2	85.6	20.2	26.3	53.5	35.2	41.2	23.6	1.3270	2.0887	1.4199	36.90	43.34	22.07
3	89.6	17.0	12.1	70.9	43.0	30.0	27.0	1.7042	2.8940	1.0288	43.42	34.00	28 37
4	80.6	44.4	39.5	16.1	56.1	31.9	12.0	1.1202	1.0204	3.0100	60.12	33.59	12.04
5	82.3	36.8	14.0	49.2	59.2	24.8	15.0	1.3599	2.1495	1.1310	57.98	26.94	17.57
6	86.2	8.1	71.9	20.0	16.0	59 0	23.0	1.4721	1.2411	3.5891	12.69	54.10	16.62
7	78.5	62.1	15.1	22.8	67.2	20.9	11.9	1.0208	2.3209	2.4627	66.21	20.05	16.79
8	76.7	82.4	9.2	8.4	85.3	8.2	5.5	1.0317	1.5855	3.2015	83.58	7.77	5.03
9	102.0	7.2	2.8	90.0	30.2	14.1	55.7	2.0052	4 0965	1.0045	32.53	17.95	55.84
10	96.5	8.8	10.2	81.0	21.4	38.5	40 1	1.7943	3.6562	1.0000	22.88	42.71	41.59
11	84.7	21.9	31.5	46.6	35.2	42.6	22.2	1.2544	1.8523	1.5890	36.54	43.70	21.49
12	88.3	16.8	17.6	65.6	38.2	36.6	25.2	1.5922	2.5334	1.5938	38.19	41.81	26.27
13	84.6	26.0	20.0	54.0	45.6	33.6	20.8	1.3738	2.3098	1.2893	46.27	27.94	20.67
14	81.0	35.6	20.0	44.4	57.2	26.4	16.4	1.4103	2.0321	1.4629	54.43	26.40	16.02
15	80.3	47.2	18.4	34.4	66.3	19 4	14.3	1.2605	1.6577	1.7077	62.07	21.00	14.10
16	81.2	36.0	40.6	23.4	48.3	34.2	17.5	1.1710	1.2890	2.9334	45.95	35.18	14.92
17	82.3	38.5	7.5	54.0	67.3	12.4	20.3	1.4886	1.4454	1.3978	63.79	13.62	17.72
18	80.2	46.0	19.0	35.0	62.0	21.2	16.8	1.2174	1.7643	1.9896	59.43	21.61	14.12
19	92.2	9.3	12.2	78.5	30.0	37.2	32.8	2.0235	3.2965	1.0113	27.56	42.86	34.09
20	93.2	10.0	12.4	77.6	27.5	35.0	37.5	1.6791	2.9667	1.1226	30.14	40.08	40.30
21	90.1	15.6	15.2	69.2	38.0	34.0	28.0	1.6232	2.5839	1.0732	38.93	35.45	28.88
22	86.1	14.5	39.5	46.0	25.6	51.2	23.2	1.3232	1.6969	1.5871	24.34	53.32	21.28
23	87.1	15.6	30.0	54.4	28.0	47.2	24.8	1.3064	1.9965	1.3741	29.60	50.68	21.46
24	84.1	28.4	18.8	52.8	51.2	29.5	19.3	1.4324	2.1905	1.2505	50.15	30.56	20.34



BENZENE — n-HEPTANE — n-BUTANOL

FIG. x — y

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VITAMINS AND NITROGEN REQUIREMENTS OF *ARTHROBACTER* SPECIES

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Abstract

Fifty-two strains of *Arthrobacter*, picked from a collection of 170 strains isolated from glycine enrichments inoculated with soil, sewage and activated sludge were found to be exacting to vitamins or aminoacids. According to their requirements they could be divided into five sub-groups. The I subgroup demanded thiamine alone, the II thiamine and pantothenate, the III thiamine, pyridoxine and glutamic acid, the IV thiamine, pantothenate and glutamic acid or sulphur containing amino acids as nitrogen source. The V subgroup comprised those which demanded organic form of nitrogen, glutamic acid serving the purpose most effectively. Biotin was found stimulatory but not essential for the II and III Subgroups. Some of the strains in subgroup II exhibited an interesting cycle of morphogenesis in media with and without biotin. A role for biotin in the normal life cycle of *Arthrobacter* has been postulated.

Introduction

According to Conn and Dimmick (1947), the soil *Arthrobacter* represent those which can utilize inorganic nitrogen in the absence of vitamins but this observation has not been substantiated by other investigators. Taylor (1938), for example, reported that only 17 out of 106 soil *Arthrobacter* were able to grow on nitrate nitrogen in the absence of yeast extract. Jensen (1952) revealed that only certain strains of soil coryneform bacteria were able to utilize inorganic nitrogen compounds while others demanded organic form of nitrogen. Morris (1960), and Chan and Stevenson (1962), also found that a good growth of *A.globiformis* can be obtained with inorganic nitrogen only when biotin was incorporated in the medium and this has subsequently been confirmed by Veldkamp *et al.* (1963). Mulder (1963) pointed out that almost all of the soil *Arthrobacter* strains he studied were able to utilize inorganic nitrogen though some demanded biotin for the purpose and others depended on a vitamin mixture or even on a vitamin mixture and casamino acids for growth to occur.

In general, it may be stated that the vitamin requirements of *Arthrobacter* have not adequately been studied. Of the few available reports, that of Lochhead and Thexton (1952) and Lochhead and Burton (1955) reveal that some of the species required thiamine alone or thiamine and biotin in addition

to vitamin B_{12}. Subsequently, Lochhead (1958) described two new species of *Arthrobacter* demanding respectively vitamin B_{12} and terregens factor and Chan (1964) reported on the biotin requirement of *A.globiformis.*

In the present paper are described details of the experiments carried out on the nutritional requirements of 52 *Arthrobacter* strains picked at random from fresh isolates made from various samples of soil, sewage and activated sludge. It would be clear from the results that organisms of this genus can conveniently be grouped into 5 distinct nutritional types, thus lending support to the conclusion of Knight and Proom (1950) and Dias and Bhat (1963, 1964) that nutritional survey could be a valuable aid in the identification of the sporeforming and diphtheroid bacteria.

Materials and Methods

Organisms: *Arthrobacter* cultures employed in this study were isolated from glycine enrichments inoculated with soils, sewage and activated sludge by the method described previously by Mullakhanbhai and Bhat (1966 b). The pure cultures were maintained on nutrient agar and glycine agar slants. Their characterization was achieved by following the techniques described in 'Manual of Microbiological Methods' Conn *et al.* (1957). Morphological and Gram staining properties were observed at intervals of 8, 18, 24 and 36 hours of growth on n. agar slants and basal salt liquid medium containing appropriate carbon and nitrogen source plus the growth factors required. Strains mentioned herein, as belonging to Group I, II and IV, were derived from soils whereas those belonging to group III were those exclusively derived from sewage and activated sludge. The group V strains again were from soil and sewage.

Media for nutritional studies: The techniques used in the elucidation of the nutritional requirements were essentially those described by Dias and Bhat (1964) except that the basal mineral base employed was that of Khambata *et al.* (1960). All tests were done in test tubes (1.8′ × 15 cm.) each containing 10 ml of the medium. Inoculum in each case was made from a 24 hour old culture (growing on n. agar slant) suspended in normal saline centrifuged at 6000 r.p.m. and washed twice and resuspended in distilled water. Aliquots of 0.05 ml of a thin suspension were inoculated and the tubes incubated at room temp. (20 – 26°C). For elucidating the vitamin nutrition, cultures were serially transferred twice and the growth response was measured on a Bausch and Lomb 'Spectornic 20' colorimeter at 540 m μ.

Results

Nutritional properties of sub-group I: The minimal requirements for the growth of sub-group I *Arthrobacter* strains in the basal salt solution containing 1% glucose and 0.05% ammonium sulphate are shown in Table I. Thiamine

TABLE I

Growth of *Arthrobacter* (Sub-group I) as affected by different vitamins

Vitamins excluded from the mixture	*Arthrobacter* strains							
	83	84	85	86	87	88	89	90
	Growth response (100 – % transmittance)							
None	40	45	50	50	40	40	45	50
Thiamine	0	0	0	0	0	0	0	0
Nicotinic acid	39	48	48	51	39	37	47	49
Calcium pantothenate	41	50	46	50	45	39	45	50
Pyridoxine	38	45	49	50	43	40	49	51
p.aminobenzoic acid	40	45	50	52	41	38	46	50
Folic acid	41	50	50	49	40	40	43	49
p.aminobenzoic acid and Folic acid	40	50	46	50	39	40	45	49
Biotin	41	48	50	50	40	40	47	50
Riboflavin	40	48	49	51	40	39	45	50
Vitamin B_{12}	40	50	50	50	40	36	43	49
All	0	0	0	0	0	0	0	0

TABLE II

Growth of *Arthrobacter* (Sub-group II) as affected by different vitamins

Vitamins excluded from the mixture	*Arthrobacter* strains						
	75	76	77	78	79	80	81
	Growth response (100 – % transmittance)						
None	50	40	38	40	50	41	39
Thiamine	0	0	0	0	0	0	0
Nicotinic acid	50	40	40	41	49	40	43
Calcium panthothenate	0	0	0	0	0	0	0
Pyridoxine	51	42	35	42	47	41	40
p.aminobenzoic acid	50	40	37	41	50	42	40
Folic acid	50	42	40	40	50	39	39
p.aminobenzoic acid and Folic acid	50	42	39	40	48	40	41
Biotin	30	28	30	32	30	40	40
Riboflavin	51	40	38	42	48	42	40
Vitamin B_{12}	51	41	40	40	48	42	40
All	0	0	0	0	0	0	0

TABLE III

Growth of *Arthrobacter* (Sub-group III) as affected by different vitamins *

Vitamins excluded from the mixture			*Arthrobacter* strains 49	50	51	52	53	54	55
			Growth response (100 – %transmittance)						
None	.....	.....	45	55	52	55	48	50	47
Thiamine	.....	.....	0	0	0	0	0	0	0
Nicotinic acid	.....	.....	43	57	50	55	47	51	49
Calcium panthothenate	.....	.....	43	55	50	53	49	50	46
Pyridoxine	.....	.....	0	0	0	0	0	0	0
p.aminobenzoic acid	.....	.....	45	50	52	55	50	49	46
Folic acid	.....	.....	45	58	50	52	48	49	49
p.aminobenzoic acid and Folic acid		.....	42	55	51	51	48	50	46
Biotin	.....	.....	45	55	52	53	50	50	45
Riboflavin	.....	.....	46	58	50	55	49	51	48
Vitamin B_{12}	.....	.....	44	55	50	55	47	50	48
All	.....	.....	0	0	0	0	0	0	0

* Basal salt solution containing 0.1% Na-glutamate as nitrogen source and 1% glucose as carbon source.

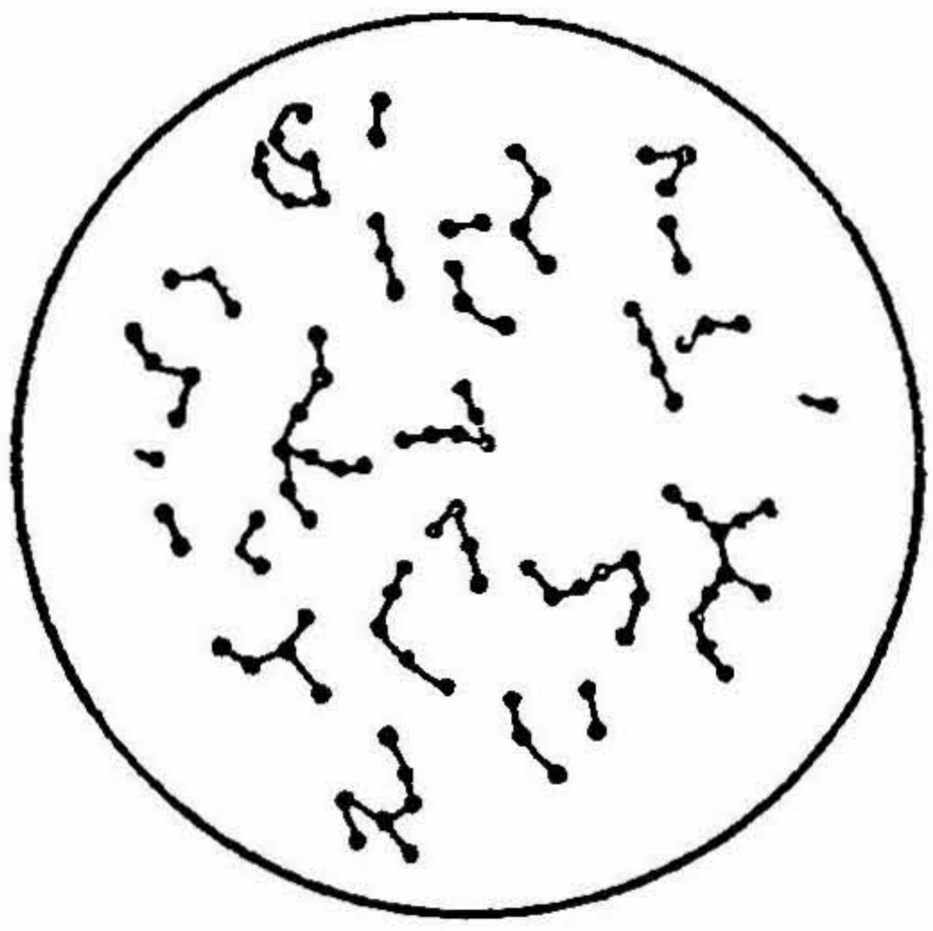
was indispensable for growth for all the strains. The suitability or otherwise of various compounds as substitutes for the essential vitamin is indicated in Table V. It is clear that the requirement for thiamine could not be met with other substitutes. Sorbitol, known for its thiamine sparing action in animal systems, could not substitute the vitamin in the bacterial system. None of the inorganic and organic nitrogenous compounds tested supported good growth (Table IX). Likewise, glutamate only was observed to be suitable as a combined source of of carbon and nitrogen to all the strains and rest of the compouuds, served only to a limited extent for strains 87, 88, 89 and 90. It is interesting to note that glutamate was the only compound that supported growth of the first four strains, *viz*., 83, 84, 85 and 86 (Table XI) and this provided a means for the demarcation of one from the other group.

Sub group II: The minimal requirements for growth of this sub-group *Arthrobacter* strains in basal salt – glucose – ammonium sulphate medium are shown in Table II. All the strains demanded thiamine and pantothenate for growth. Other compounds when tested for their suitability as substitute for the essential vitamins (Table VI) revealed that none could replace either

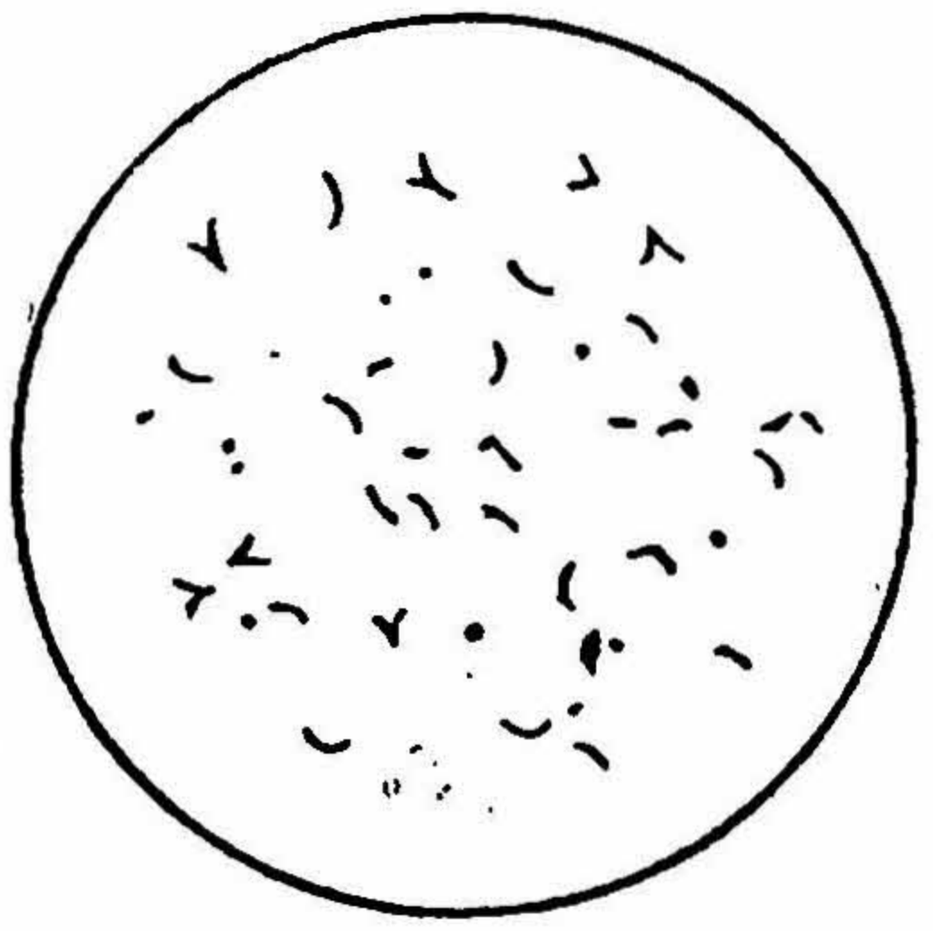
thiamine or pantothenate. Of interest is the finding that β-alanine could not replace pantothenate and that biotin was growth stimulatory but not essential. However, biotin deficient medium promoted the formation of abnormal forms (see Fig. I) and led to the accumulation of intracellular inclusions. In fact, in the absence of biotin the cell division was interrupted and growth was retarded whereas in the presence of biotin the normal life cycle was completed. The ability of this subgroup strains to utilize various nitrogen compounds is shown in Table X. Ammonium sulphate proved to be a good source of nitrogen. All the amino-acids tested supported growth, asparagine and cysteine proving themselves to be exceptionally good sources. Of the various compounds tested as combined sources of nitrogen and carbon (Table XII), aspartic acid and asparagine supported fairly good growth whereas alanine and glutamate were excellent. Of interest is the observation that sulphur containing amino acids served as satisfactory nitrogen sources but not as sources of combined nitrogen and carbon.

Sub-group III: The minimal requirements for growth of subgroup III *Arthrobacter* strains in basal salt solution + 1% glucose and 0.1% glutamate are presented in Table III. Pyridoxine and thiamine were indispensable as growth factors to all the strains. In the absence of vitamins even a trace of growth was not observed in first transfer indicating thereby that they had an absolute demand for these growth factors. Table VII represents the growth response of the cultures in media supplemented with various substances. None was found suitable to replace thimaine and pyridoxine. Biotin was found stimulatory but not essential for growth and the demand seemed more

Effect of Biotin on the morphogenesis of *Arthrobacter Sp.* Sub-group II



4 Days old Cells
from medium without Biotin
showing a stressed condition
with granules, deeply stained



4 Days old Cells
from medium containing Biotin
showing pleomorphic rods and
Cocci (Normal Life Cycle)

FIG. I

conspicuous when the organisms were cultivated in 0 5% glutamate containing medium. Glutamate was the only compound that served well as a source of nitrogen, as well as a combined source of carbon and nitrogen, but even in its presence the damand for thiamine and pyridoxine was very sharp and unequivocal suggesting thereby the suitability of the strains for the detection and assay of such factors (Mullakhanbhai and Bhat, 1966 *a*).

Sub-group IV: The need for growth factors by these *Arthrobater* strains in basal salt solution + 1% glucose and 0.1% glutamate is shown in Table IV. With all strains it was observed that thiamine and pantothenate were indispensable for growth. Like those in the previous subgroups the strains in this subgroup also could not utilize substitutes of the two vitamins (Table VIII). Glutamate and two sulphur containing aminoacids however were suitable as N. sources. Glutamate was also suitable as a combined source of nitrogen and carbon.

Sub-group V: All the strains in this subgroup demanded organic form of nitrogen but could do without any growth factor. Glutamate was the only compound found to be the most suitable substrate for growth, though complex substrates like peptone and casein-hydrolysate also supported their good growth.

TABLE IV

.Growth of *Arthrobacter* (Sub-group IV) as affected by different vitamins *

Vitamins excluded from the mixture	*Arthrobacter* strains						
	67	68	69	70	71	72	73
	Growth response (100 – % transmittance)						
None	55	48	50	45	52	50	40
Thiamine	0	0	0	0	0	0	0
Nicotinic acid	55	48	50	47	50	50	42
Calciam pantothenate	0	0	0	0	0	0	0
Pyridoxine	53	47	51	47	52	51	40
p aminobenzoic acid	53	49	50	44	52	51	41
Folic acid	55	48	50	45	51	50	40
p.aminobenzoic acid and Folic acid	55	48	52	47	50	50	42
Biotin	54	49	50	45	50	52	40
Riboflavin	55	47	51	45	52	50	41
Vitamin B_{12}	55	47	50	45	50	50	41
All	0	0	0	0	0	0	0

* Basal salt solution containing 0.1% Na-glutamate as nitrogen source and 1% glucose as carbon source.

TABLE V

Growth response of *Arthrobacter* (Sub-group I) in various media

Supplements added to medium	*Arthrobacter* strains							
	83	84	85	86	87	88	89	90
	Growth response (100 – % transmittance)							
Vitamin mixture	40	47	50	50	42	40	46	50
Thiamine	35	40	46	46	37	35	40	39
Thiamine + Nicotinic acid	37	40	44	47	38	32	40	38
Thiamine + Calcium pantothenate	35	39	45	47	36	34	41	38
Thiamine + pyridoxine	38	40	45	45	37	34	40	39
Thiamine + Folic acid	40	41	43	47	36	36	40	40
Thiamine + *p*.aminobenzoic acid	40	40	45	45	36	35	38	42
Thiamine + Biotin	39	39	47	45	35	38	38	49
Thiamine + Riboflavin	35	39	46	47	37	34	37	40
Thiamine + Vitamin B_{12}	36	40	48	46	37	35	38	41
Thiamine + Casein hydrolysate	42	41	50	46	40	40	42	50
Thiamine + Asparagine	36	38	46	44	38	38	37	41
Thiamine + Glutamate	38	38	48	47	38	38	38	43
Thiamine + Aspartic acid	35	38	48	47	63	37	36	42
Methionine + Sorbitol	0	0	0	0	0	0	0	0
Sorbitol + Casein-hydrolysate	0	0	0	0	0	0	0	0
Biotin + Casein-hydrolysate	0	0	0	0	0	0	0	0
Biotin + Sorbitol	0	0	0	0	0	0	0	0
None	0	0	0	0	0	0	0	0

DISCUSSION

The observation that species in the genus *Arthrobacter* occur in a wide variety of material and are almost ubiquitous is suggestive of their importance in nature. Though the type species *Arthrobacter globiformis* is simple in its nutritional requirement other species studied here (as well as by others elsewhere) seem to demand specific growth factors and that this demand is so absolute and specific as to suggest the exploitation of the species for the detection and assay of growth factors present in biological materials. It is also apparent from the above results that the requirements for essential metabolites or growth factors are characteristics for most of the species within a genus eventhough certain strains within a species may behave differently.

The morphology of some bacterial cultures grown under laboratory cultural conditions is greatly influenced by the components of the medium in which they are grown. Cultivation of an organism in a medium limiting in one or more components may likewise induce an 'abnormal' morphogenesis. This was clearly observed in some strains of *Arthrobacter* subgroup II where biotin deficiency led to a condition in which cells failed to fragment thereby retarding growth. Bitoin deficiency also caused the accumulation of intracellular inclusions (deeply stained granules) whereas biotin sufficiency restored normalcy in their life cycle. Such deformities in the morphogenesis under deficient conditions have previously been observed by Chan (1964) in *A.globiformis.*

TABLE VI

Growth response of *Arthrobacter* (Sub-group II) in various media

Supplements added to medium	*Arthrobacter* strains						
	75	76	77	78	79	80	81
	Growth response (100 – % transmittance)						
Vitamin mixture	50	40	35	35	47	45	42
Thiamine + Calcium pantothenate	50	39	35	35	45	45	40
Thiamine	0	0	0	0	0	0	0
Calcium pantothenate	0	0	0	0	0	0	0
Thiamine + Biotin	0	0	0	0	0	0	0
Thiamine + Pyridoxine	0	0	0	0	0	0	0
Thiamine + Vitamin B_{12}	0	0	0	0	0	0	0
Calcium pantothenate + Biotin	0	0	0	0	0	0	0
Thiamine + β-alanine	0	0	0	0	0	0	0
Thiamine + Asparagine	0	0	0	0	0	0	0
Thiamine + Glutamate	0	0	0	0	0	0	0
Thiamine + Casein-hydrolysate	0	0	0	0	0	0	0
Thiamine + Valine + Leucine	0	0	0	0	0	0	0
Sorbitol + Calcium pantothenate	0	0	0	0	0	0	0
Thiamine + Sorbitol	0	0	0	0	0	0	0
None	0	0	0	0	0	0	0

TABLE VII

Growth response of *Arthrobacter* (Sub-group III) in various media

Supplements added to medium	*Arthrobacter* strains						
	49	50	51	52	53	54	55
	Growth response (100 – % transmittance)						
Vitamin mixture	45	50	52	50	45	50	50
Thiamine	0	0	0	0	0	0	0
Pyridoxine	0	0	0	0	0	0	0
Thiamine + Pyridoxine	40	46	45	44	40	45	45
Thiamine + Pyriodine + Biotin	46	50	50	49	46	48	48
Thiamine + Asparatic acid	0	0	0	0	0	0	0
Thiamine + γ.aminobutyric acid	0	0	0	0	0	0	0
Thiamine + Asparagine	0	0	0	0	0	0	0
Thiamine + Casein-hydrolysate	0	0	0	0	0	0	0
Sorbitol + Pyridoxine	0	0	0	0	0	0	0
Thiamine + Sorbitol	0	0	0	0	0	0	0
Pyridoxine + Methionine	0	0	0	0	0	0	0
None	0	0	0	0	0	0	0

Taxonomical consideration. The genus *Arthrobacter*, Conn and Dimmick (1947) was created with a view to segregate the soil " diphtheroids " from the true corynebacteria comprising primarily plant or animal pathogens. The *Arthrobacter*, in true sense, are saprophytes. They differ from *Corynebacterium* species in possessing a more complicated life cycle, in the course of which often short branched filamentous rods appear. The latter characteristic renders the boundary between *Arthrobacter* and *Nocardia* less distinct. However, in *Nocardia* branching is more persistent whereas in *Arthrobacter* true branching does not occur (Skerman, 1959) though occasionally rudimentary budding might be observed. A detailed study of cytology cellular morphology and cell-wall composition of the three genera may reveal some clear-cut demarcations between them and this aspect is under investigation in this laboratory.

In order not to complicate the taxonomy of *Arthrobacter* the authors do not intend to propose new names for the species worked out here. Detailed taxonomic studies have indeed been conducted in this laboratory and a simplified scheme will be proposed in the near future to make the taxonomic position of *Arthrobacter* more clear.

TABLE VIII

Growth response of *Arthrobacter* (Sub-group IV) in various media

Supplements added to medium	*Arthrobacter* strains						
	67	68	69	70	71	72	73
	Growth response (100 – % transmittance)						
Vitamin mixture	55	48	50	43	50	50	45
Thiamine	0	0	0	0	0	0	0
Calcium pantothenate	0	0	0	0	0	0	0
Thiamine + Calcium pantothenate	51	44	49	40	48	49	40
Thiamine + Calcium pantothenate + Biotin	53	47	49	32	50	48	42
Thiamine + Biotin	0	0	0	0	0	0	0
Thiamine + Pyridoxine	0	0	0	0	0	0	0
Thiamine + Vitamin B_{12}	0	0	0	0	0	0	0
Calcium pantothenate + Biotin	0	0	0	0	0	0	0
Thiamine + β-alanine	0	0	0	0	0	0	0
Thiamine + Asparatic acid	0	0	0	0	0	0	0
Thiamine + Asparagine	0	0	0	0	0	0	0
Thiamine + Casein-hydrolysate	0	0	0	0	0	0	0
Thiamine + Valine + Leucine	0	0	0	0	0	0	0
Thiamine + Sorbitol	0	0	0	0	0	0	0
Sorbitol + Calcium pantothenate	0	0	0	0	0	0	0
None	0	0	0	0	0	0	0

TABLE IX

Growth response of *Arthrobacter* (Sub-group I) in media containing different nitrogen sources

Nitrogen source	*Arthrobacter* strains							
	83	84	85	86	87	88	89	90
	Growth response (100 – % transmittance)							
None	0	0	0	0	0	0	0	0
Sodium nitrite	0	0	0	0	0	0	0	0
Sodium nitrate	41	34	15	0	0	0	0	44
Ammonium sulphate	35	38	36	39	35	35	37	35
Ammonium nitrate	20	18	22	20	15	15	21	20
Ammonium phosphate (Dibasic)	39	30	48	38	20	15	15	42
Aspartic acid	41	35	41	43	23	22	16	34
Asparagine	25	18	32	30	15	16	18	46
Arginine	30	30	41	36	18	18	29	51
Alanine	36	25	44	45	28	25	25	57
β-alanine	21	20	24	27	17	50	20	17
Cysteine	42	40	39	41	38	32	30	33
Glycine	20	20	26	26	18	15	18	15
Glutamate	41	45	58	59	27	22	30	48
Histidine	45	23	62	56	15	15	15	50
Lysine	32	30	41	35	0	0	0	37
Leucine	30	28	40	46	18	67	20	30
Methionine	22	20	24	28	18	25	18	20
Proline	0	15	15	18	0	0	0	29
Phenylalanine	28	20	35	29	15	15	15	20
Serine	25	21	30	27	0	0	0	0
Threonine	26	20	27	29	37	50	30	20
Valine	26	25	35	34	0	0	0	17
Urea	0	0	0	0	0	0	0	0
Urate	0	0	0	0	0	0	0	0
Creatinine	0	0	0	0	0	0	0	0
Peptone	31	30	35	27	38	31	30	60
Casein-hydrolysate	24	30	35	30	22	20	20	56

TABLE X

Growth response of *Arthrobacter* (Sub-group II) in media containing different nitrogen sources

Nitrogen Source	*Arthrobacter* strains						
	75	76	77	78	79	80	81
	Growth response (100 – % transmittance)						
None	0	0	0	0	0	0	0
Sodium nitrite	0	0	0	0	0	0	0
Sodium nitrate	18	0	0	0	18	15	19
Ammonium sulphate	50	40	35	35	47	49	51
Ammonium nitrate	27	25	15	20	26	25	27
Ammonium phosphate (Dibasic)	35	40	20	20	34	36	35
Aspartic acid	21	31	25	17	20	20	21
Asparagine	46	47	26	17	45	44	47
Arginine	16	51	20	28	15	15	15
Alanine	18	51	21	25	18	18	20
β-alanine	30	26	40	45	29	30	28
Cysteine	50	15	40	42	47	44	40
Glycine	18	15	16	15	18	20	20
Glutamate	26	33	25	20	25	25	25
Histidine	0	50	20	15	0	0	0
Lysine	0	38	15	0	0	0	0
Leucine	15	51	20	26	15	15	15
Methionine	36	15	20	23	30	29	27
Proline	0	0	16	15	0	0	0
Phenylalanine	26	15	0	0	20	18	15
Serine	0	25	0	0	0	0	0
Threonine	34	21	40	43	30	32	30
Valine	15	0	0	0	15	15	15
Urea	0	0	0	0	0	0	0
Urate	0	0	0	0	0	0	0
Creatinine	0	0	0	0	0	0	0
Peptone	52	64	36	30	50	52	54
Casein-hydrolysate	44	60	30	20	45	45	46

TABLE XI

Suitability of compounds to serve as combined sources of carbon and nitrogen for *Arthrobacter* (Sub-group I)

Substrates			*Arthrobacter* strains 83	84	85	86	87	88	89	90
			Growth response (100 – % transmittance)							
None	.....	.....	0	0	0	0	0	0	0	0
Aspartic acid	.....	.....	0	0	0	0	65	43	50	50
Asparagine	.....	.....	0	0	0	0	22	31	25	68
Arginine	.....	.....	0	0	0	0	15	24	20	80
Alanine	.....	.....	0	0	0	0	36	26	32	41
β-alanine	.....	.....	0	0	0	0	15	0	15	39
Cysteine	.....	.....	0	0	0	0	0	0	0	0
Glycine	.....	.....	0	0	0	0	0	0	0	0
Glutamate	.....	.....	60	60	64	62	66	59	60	75
Histidine	.....	.....	0	0	0	0	58	62	55	70
Lysine	.....	.....	0	0	0	0	26	27	24	78
Leucine	.....	.....	0	0	0	0	35	32	30	0
Methionine	.....	.....	0	0	0	0	0	0	0	0
Proline	.....	.....	0	0	0	0	35	55	30	0
Phenylalanine	.....	.....	0	0	0	0	0	0	0	0
Serine	.....	.....	0	0	0	0	24	21	20	0
Threonine	.....	.....	0	0	0	0	0	0	0	0
Tryptophane	.....	.....	0	0	0	0	0	0	0	0
Valine	.....	.....	0	0	0	0	0	0	0	0
Urea	.....	.....	0	0	0	0	0	0	0	0
Urate	.....	.....	0	0	0	0	0	0	0	0
Creatinine	.....	.....	0	0	0	0	0	0	0	0
Hippurate	.....	.....	0	0	0	0	0	0	0	0
Peptone	.....	.....	56	45	46	58	61	43	60	64
Casein-hydrolysate	.....	.....	45	45	48	60	21	40	40	61

TABLE XII

Suitability of compounds to serve as combined sources of carbon and nitrogen for *Arthrobacter* (Sub-group II)

Substrates			*Arthrobacter* strains						
			75	76	77	78	79	80	81
			Growth response (100 – % transmittance)						
None	----	.....	0	0	0	0	0	0	0
Aspartic acid	.....	—	40	50	56	46	42	38	44
Asparagine	.....	.....	55	72	33	17	50	46	49
Arginine	----	----	20	88	20	0	18	20	20
Alanine	----	.....	72	62	23	16	76	70	73
β-alanine	.....	.....	0	56	0	0	0	0	0
Cysteine	----	.....	0	0	0	0	0	0	0
Glycine	.....	----	0	0	0	0	0	0	0
Glutamate	----	—	80	82	32	15	78	75	80
Histidine	----	.....	0	62	54	42	0	0	0
Lysine	----	----	0	75	28	16	0	0	0
Leucine	—	—	0	0	24	26	0	0	0
Methionine	.....	.....	0	0	0	0	0	0	0
Proline	----	.....	26	32	49	46	27	25	29
Phenylalanine	.....	.....	0	0	0	0	0	0	0
Serine	----	.....	0	0	27	0	0	0	0
Threonine	.....	.....	0	0	0	0	0	0	0
Tryptophane	.....	.....	0	0	0	0	0	0	0
Valine	.....	----	0	0	0	0	0	0	0
Urea	—	.....	0	0	0	0	0	0	0
Urate	----	.....	0	0	0	0	0	0	0
Creatinine	.....	----	0	0	0	0	0	0	0
Hippurate	.....	.....	0	0	0	0	0	0	0
Peptone	----	.....	77	75	44	37	70	69	60
Casein-hydrolysate	.....	.....	68	75	29	32	66	65	57

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