Fig. 2. The partition quotient ϕ as a function of the ketone concentration in the organic phase.

○: $\text{Co(NH}_3)_6^{3+}$; $C_B = 0.67$ M; left ordinate scale.
 ●: $\text{Co(NH}_3)_6^{3+}$; $C_B = 0.80$ M; left ordinate scale.
 ●: $\text{Co en}_2(\text{NCS})_2^+$; $C_B = 0.67$ M; right ordinate scale.

as some cyclohexanone dissolves in the aqueous phase (cf. Ref.², p. 709).

Analogous measurements were also performed by using the *trans*- $\text{Co en}_2(\text{NCS})_2^+$ ion as the inert cobalt complex ion. Then the extracted species contains probably only one salicylate ion and more space should be available for the ketonic ligands. The cobalt concentration in the two phases was determined colorimetrically. The results are given in Figs. 1 and 2. $C_B = 0.67$ M was used for the measurements of Fig. 2. It is easily deduced that eqn. 5 should also apply here. In Fig. 3 $(\phi/\phi_0 - 1) [K]^{-1}_{\text{org}}$ is plotted against $[K]_{\text{org}}$. The linearity indicates the presence of two complexes. $\beta_{1 \text{ org}} = (0.76 \pm 0.05) \text{ M}^{-1}$ and $\beta_{2 \text{ org}} =$

Fig. 3. $X_1 = (\phi/\phi_0 - 1) [K]^{-1}_{\text{org}}$ plotted against $[K]_{\text{org}}$.

$(0.24 \pm 0.05) \text{ M}^{-2}$. Also here deviation from linearity occurs at high $[K]_{\text{org}}$.

If we assume that each salicylate ion uses two coordination sites, which is likely but not quite certain, at least seven coordination sites are filled in the outer sphere of the $\text{Co(NH}_3)_6^{3+}$ ion and four sites in that of the $\text{Co en}_2(\text{NCS})_2^+$ ion.

It is interesting to note that the coordination number found in this case, for the cobalthexammine ion is very near the number eight expected from geometrical considerations. In the anionic complex discussed above, we should indeed have an example of outer-sphere coordination number eight. It is also interesting to note the similar magnitude of $\beta_{1 \text{ org}}$ for the two central groups studied. The possibility of an association of ketones with cobaltamines has been suggested earlier³ from other measurements.

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Pteroylglutamic Acid as a Growth Factor for a Strain of *Rhodotorula*

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In a study of fungi occurring on slime-flux of tree stumps, a strain of *Rhodotorula*, called 18 b, was isolated. This appeared to require *p*-aminobenzoic acid (PAB) as an essential factor for growth¹. Since such a requirement is extremely rare among fungi, the nutrition of strain No. 18 b was more closely studied. This strain does not belong to *Rhodotorula aurantiaca*, the PAB-requirement of which has been investigated by Robbins and Ma², nor does it seem to be identical with any other earlier described species of the genus *Rhodotorula*.

The fungus was cultivated in continuously shaken test tubes with 10 ml of a synthetic liquid medium, A 2 + B, containing glucose, ammonium phosphate, and mineral salts³. For growth in this medium the fungus also required, apart from PAB, an addition of thiamine (100 $\mu\text{g/l}$). The growth was estimated photometrically at 610 $m\mu$ with an Engel colorimeter (Kipp, Delft). Extinction values were expressed according to the formula, $Z = (e_n - e_0) \times 10^3$, where e_0 represents the original extinction value of the culture after inoculation and e_n the value obtained after an incubation period of n hours. Each extinction value represents an average of determinations for three tubes. The incubation temperature was 25°C and the initial pH was 6.4.

Experiments with different concentrations of PAB showed that the strain responded to a 10^{-9} M solution of PAB, i.e. 0.0014 μg per 10 ml nutrient medium, a sensitivity which is about the same as that of previously studied PAB-heterotrophic organisms. However, an unexpectedly high concentration (~ 0.75 μg per 10 ml) was necessary for a maximum rate of growth, a fact which might indicate a partial deficiency for other growth factors (Table 1).

In contrast to, e.g., certain strains of *Saccharomyces cerevisiae*⁴ our PAB-requiring strain of *Rhodotorula* is able to utilize pteroyl glutamic acid (PtG) instead of

Table 1. Response of *Rhodotorula* 18 b to *p*-aminobenzoic acid (PAB) and pteroylglutamic acid (PtG). 3.2 μg of PtG is equivalent to 1 μg of PAB

Expt. No.	PAB $\mu\text{g/l}$	PtG $\mu\text{g/l}$	Growth expressed as extinction values, Z , obtained after		
			75 h	140 h	168 h
10	0	0	40	—	70
	0.1	0	110	—	280
	1	0	190	—	510
	10	0	240	—	840
	50	0	310	—	1340
	100	0	360	—	1280
	200	0	330	—	1220
11	0	0	30	50	—
	100	0	480	1180	—
	0	320	180	340	—
	0	16 000	500	1160	—
	0	32 000	480	1160	—

Table 2. The inhibiting effect of sulphadiazin on *Rhodotorula* 18 b grown with PAB or PtG. Incubation time 96 h.

Addition to the medium			Extinction value, Z , of the culture
PAB $\mu\text{g/l}$	PtG $\mu\text{g/l}$	Sulphadiazin mg/l	
0	—	0	70
100	—	0	850
100	—	1	710
100	—	10	430
100	—	30	170
100	—	100	10
1000	—	0	740
1000	—	10	480
1000	—	100	10
—	16 000	0	730
—	16 000	10	330
—	16 000	100	10

PAB (Table 1). Ten to fifty times as much PtG as PAB (in terms of moles) is necessary in order to produce the same growth response. In this respect *Rhodotorula* 18 b is like *Lactobacillus arabinosus*, *L. plantarum*⁵ and *Clostridium acetobutylicum*⁶.

The possibility that the effect of PtG was due to contamination by PAB was tested. A chromatographical analysis, with butanol saturated with 1.5 N NH_4OH as the solvent⁷ and with methyl red as the colour reagent, showed that the preparation of PtG used contained less than 0.6 % PAB. Thus, *Rhodotorula* 18 b was able to utilize PtG as such.

In the great majority of cases, so far known, where PtG can be used as well as PAB, the growth-inhibiting effect of sulphadiazin disappears when PAB is replaced by PtG (or its derivative leucovorin). *Rhodotorula* 18 b, however, is also inhibited by sulphadiazin when grown with PtG (Table 2). This may indicate that PtG is not directly incorporated, but that it is first broken down, and only the PAB part is utilized.

Finally, the possibility was investigated that PAB could be entirely or partially substituted by a mixture of amino acids and purines. This had been demonstrated in some earlier investigated cases (by, e.g., Cutts and Rainbow⁸, and Aaronson and

Table 3. The sparing effect of some nucleic acid components and amino acids on the requirement for PAB in *Rhodotorula* 18 b. AN-mixture: L-methionine 10 mg/l, DL-histidine 20 mg/l, L-leucine 10 mg/l, L-serine 10 mg/l, and thymine 30 mg/l.

PAB $\mu\text{g/l}$	Additions to the medium		Extinction value, Z, of the culture after	
	AN-mix- ture + adenosi- ne, 20 mg/l	AN-mix- ture + inosinic acid, 29 mg/l	104 h	144 h
0	—	—	250	350
1	—	—	290	400
10	—	—	450	660
50	—	—	710	1070
100	—	—	870	1260
0	+	—	300	370
1	+	—	520	680
10	+	—	700	970
50	+	—	870	1300
100	+	—	840	1290
0	—	+	330	450
1	—	+	480	690
10	—	+	570	880
50	—	+	880	1380
100	—	+	820	1320

Rodriguez⁹). Preliminary tests with thymine, amino acids and various purines, alone and combined, showed that a mixture of adenosine, thymine and four amino acids gave the highest rate of growth in the presence of PAB (Table 3). This mixture was not a substitute for PAB, but exerted a sparing action, so that half maximum growth rate was attained with approximately 1 μg instead of 10 μg of PAB per liter. Adenosine could be exchanged for inosinic acid with no appreciable loss of activity of the mixture.

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Observation of Proton Magnetic Resonance Shifts in H_2O - D_2O Mixtures

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Isotope effects on nuclear magnetic resonance have been reported for specific groups in organic compounds. Carbon and hydrogen isotope effects have been observed in fluorine and proton magnetic resonance (PMR). In all the investigations the resonance of observed nuclei in a group containing a heavy isotope of the same or another element was displaced upfield relative to the resonance in a group with a light isotope. The present note reports a PMR study of H_2O - D_2O mixtures. PMR shifts were determined at 60 Mc/sec both by internal and external reference methods. Recently the PMR spectrum of the HDO and H_2O species in an acetone solution was reported¹; the HDO resonance signal, a triplet due to the H-D spin-spin coupling, was observed at a slightly higher field than the singlet line of H_2O .

Experimental. The PMR spectra were recorded with a Varian Associates A-60 spectrometer, operating at the fixed frequency of 60 Mc/sec. The resonance line shifts were determined by means of side band technique using a Hewlett Packard Model 202 A audio oscillator. The determination of the frequency was carried out by means of a Model 5512 A electronic

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