RESULTS

Twenty-five consecutive specimens sent to the laboratory for fat determination, from patients in the Leeds General Infirmary, have been analyzed by this method and by the wet extraction method. The results on 23 of the specimens are analyzed in Table 1.

Table 1. *Fat content of 23 stools by plaster of Paris method and wet extraction method*

	Total fat in dry faeces		Split fat in dry faeces		Unsplit fat in dry faeces	
	Plaster method (%)	Wet extraction method (%)	Plaster method (%)	Wet extraction method (%)	Plaster method (%)	Wet extraction method (%)
Range	3.52-51.54	4.86-51.48	3.06-48.39	3.15-46.81	0.13-30.12	nil-30.97
Average	28.06	28.68	23.40	23.26	4.66	5.42
Standard error of difference between methods	4.03		4.07		1.71	

DISCUSSION

In all but two cases agreement between the results by the two methods was highly satisfactory, having regard to the nature of the material analyzed. Moreover, such differences as existed were not all in one direction, so that there is no indication that one method offers a more efficient method of fat extraction than the other.

The two specimens which showed less satisfactory agreement were very watery specimens. Frequently such specimens contain material which separates out from the rest very readily, so that it is hardly possible to maintain uniformity of composition sufficiently long to weigh out the separate portions required for analysis by the two methods. Differences in the figures in such cases are more likely to

represent a difference in the composition of the material analyzed than a disagreement between the two methods.

In no case (including the two specimens just referred to) was there any difference in clinical significance between the figures by the two methods.

The plaster method as described gives, therefore, substantially the same results as the wet extraction

method, and on account of the very real saving of labour its adoption is recommended.

SUMMARY

1. A method for the determination of split and unsplit fat in faeces, by drying the specimen with plaster of Paris, extraction with light petroleum in a Soxhlet apparatus and subsequent titration of the extracted fat has been described. Probable sources of error have been dealt with.

2. Comparison of the results by this method with those of a wet extraction method previously in use shows that the plaster of Paris method is efficient and satisfactory. Owing to the saving of labour involved, the plaster method is to be preferred to the wet extraction method.

REFERENCE

Fowweather, F. S. (1926). *Brit. J. exp. Path.* 7, 7.

The Solubilization of Polycyclic Aromatic Hydrocarbons by Purines

By H. WEIL-MALHERBE, *Cancer Research Laboratory, North of England Council of the British Empire Cancer Campaign, Royal Victoria Infirmary, Newcastle upon Tyne*

(Received 1 January 1946)

Brock, Druckrey & Hamperl (1938) found that 3:4-benzpyrene was appreciably soluble in aqueous solutions of caffeine. They observed a similar solvent effect with theophylline and theobromine and even with xanthine. The solutions of benzpyrene in aqueous caffeine solutions possessed the same

characteristic violet fluorescence as solutions of benzpyrene in organic solvents. This indicated a true molecular dispersion, since benzpyrene either in solid form or in fine suspension or in colloidal solution has a yellow fluorescence. Joan Keilin (1943), who investigated the effect of caffeine on

haematin and porphyrins, also came to the conclusion that it had essentially a solvent action; caffeine was shown to react not with the Fe^{++} of haem, but with its porphyrin. Out of a large number of purines tested, including theobromine, theophylline and 1:3:7-trimethyluric acid, only caffeine and 8-chlorocaffeine were active; on the other hand, pilocarpine behaved with porphyrins in the same way as caffeine.

The solvent effect of caffeine is remarkable in several respects: caffeine is neither an electrolyte nor is it highly surface-active, and it does not form colloidal solutions. It thus differs from any of the known solubilizers, such as bile salts, soaps, Turkey red oil, etc. The solvent effect of caffeine is therefore of sufficient interest in itself to deserve a closer quantitative study, quite apart from the disagreement between the findings of Brock *et al.* (1938) and J. Keilin (1943) as to the solvent effect of theophylline, theobromine and other purines. To the biochemist the close connexion between caffeine and other purine derivatives of physiological importance suggests that these solvent effects may have biological significance. Finally, it might be of great value in many fields of biological research to find solubilizers which are at the same time highly active and comparatively non-toxic.

A quantitative study has therefore been made of the solvent effect of purines on 3:4-benzpyrene and other hydrocarbons, in the hope of throwing some light on the nature of the forces underlying it. A fairly large number of purines and purine derivatives was examined to analyze the influence of chemical constitution.

EXPERIMENTAL

Analytical methods. Purine solutions were shaken with an excess of solid hydrocarbon for 3-4 hr., either at room temperature (18-20°) or in a thermostat at 38°. They were then filtered through a fine filter paper (Whatman no. 5). The filtrate was examined in filtered ultra-violet light; any suspended particles which had passed through the filter were revealed as luminous points with white or yellow fluorescence which stood out vividly from the blue-fluorescent or non-fluorescent solution. If any suspended material was present, the solution was passed through a layer of kieselguhr on a Büchner funnel. However, this was necessary in only a few cases.

The clear filtrate or a suitable fraction of it was then extracted 5 times with $\frac{1}{10}$ vol. light petroleum. The combined extracts were dried over CaCl_2 and, if necessary, concentrated to 40-50 ml. They were then passed through a column of activated alumina of 4 mm. diameter containing 2 g. of adsorbent. After elution with 15 ml. benzene the solution was analyzed fluorimetrically in an atmosphere of pure nitrogen using a direct-reading fluorimeter (cf. Weil-Malherbe, 1944). The readings were calibrated by standard solutions of known strength. Care was taken to ensure that the solutions to be measured were sufficiently dilute, so that the fluorescence intensity was proportional to the con-

centration. The concentration range most suitable for fluorimetry varied from hydrocarbon to hydrocarbon according to the different fluorescence intensities of their solutions. The solutions of strongly fluorescent hydrocarbons contained from 0.1 to 1.0 $\mu\text{g./ml.}$, whereas higher concentrations (10-50 $\mu\text{g./ml.}$) had to be used with weakly fluorescent hydrocarbons, such as phenanthrene, pyrene, chrysene or 1:2:5:6-dibenzanthracene.

As several hydrocarbons, i.e. 20-methylcholanthrene, 1:2:5:6-dibenzanthracene and coronene, were not sufficiently soluble in light petroleum, they were extracted with benzene. The benzene extracts, after drying, were concentrated to 15 ml. and passed through alumina columns. The filtrates were directly used for fluorimetry.

Correction for solubility in water. The solubility of the hydrocarbons tested in pure water was determined and all figures corrected accordingly. Some of the results are contained in Table 1.

Table 1. *Solubility of some hydrocarbons in distilled water*

	Conc. of sat. solution ($\mu\text{mol.}$)
Phenanthrene	15
Chrysene	3.4
Pyrene	1.0
Anthracene	0.5
3:4-Benzpyrene	0.024

Molecular ratios. The ratio mol. purine in solution/mol. hydrocarbon in solution indicates the number of purine molecules necessary to dissolve 1 mol. of hydrocarbon and thereby the excess of purine over hydrocarbon. The 'solvent power' of a purine is the reciprocal of the molecular ratio.

Melting-points are uncorrected.

Materials. The following purines were received from Prof. A. R. Todd, F.R.S.: 3-, 7- and 9-methylxanthines, theophylline-7-d-galactoside, 2-methylthioadenine, 2-methyladenine, 2:9-dimethyladenine, 2-methylthio-9-methyladenine, 2-methylhypoxanthine, 2-methylthio-9-methylhypoxanthine. Paraxanthine was prepared by Dr F. G. Mann and was obtained through the courtesy of Miss J. Keilin.

Other purines and allied compounds were prepared according to the following methods: 8-chlorotheobromine (Biltz & Topp, 1911); 8-methoxycaffeine (Fischer, 1884); 8-ethoxycaffeine (Fischer, 1882); 8-chlorocaffeine (Fischer & Reese, 1883); 1:3-dimethyluric acid (Biltz & Heyn, 1921); 3:7-dimethyluric acid (Fischer, 1895); 3:9-dimethyluric acid (Biltz & Krzikalla, 1921, 1927); 7:9-dimethyluric acid (Biltz & Bülow, 1921a); 1:3:7-trimethyluric acid (Fischer, 1882); 1:3:9- and 3:7:9-trimethyluric acid (Biltz & Pardon, 1930); 1:7:9-trimethyl-8-thiouric acid (Biltz & Bülow, 1921b); 1:3:7:9-tetramethyluric acid (Biltz & Strufe, 1916); 1:3:7:9-tetramethyl-8-thiouric acid (Biltz & Heidrich, 1921); caffeine methiodide and methochloride and 8-hydroxy-9-methyl-8:9-dihydrocaffeine (Schmidt & Schilling, 1885); 7:9-dimethyldeoxyuric acid (Biltz & Bülow, 1921a); 1:3:7:9-tetramethyldeoxyuric acid (Biltz & Heidrich, 1921); 3:7-dimethyl-4:5-dihydroxydihydrouric acid (Biltz & Damm, 1914); 1:3:7-trimethyl-4:5-dimethoxydihydrouric acid (Biltz & Heyn, 1916)

RESULTS

The effect of purine concentration on the solubility of 3:4-benzpyrene

If the concentration of purine were linearly proportional to the concentration of benzpyrene in solution, the solvent power as expressed by the molecular ratio would be a constant, independent of purine concentration. Such a linear relationship exists, however, only at very low purine concentrations. As soon as the concentration of benzpyrene in solution reaches a certain level, i.e. $0.16 \mu\text{mol.}$, there is an abrupt change. Beyond this point the

benzpyrene concentration changes in such a way that it is roughly proportional to the square of the purine concentration. If the concentration of benzpyrene is plotted against the concentration of purine on a logarithmic scale the points lie on a straight line whose slope = the exponent f in the equation: $k = C_P^f / C_{HC}$, where C_{HC} = the concentration of hydrocarbon, C_P = the concentration of purine and k = a constant. The exponential factor f can be determined by calculating the linear regression coefficient of $\log C_{HC}$ on $\log C_P$, or, more simply, by drawing the curve and measuring its slope graphically. In every case so far investigated, in-

Table 2. *The influence of purine concentration on the solubility of 3:4-benzpyrene*

Purine	Conc. of purine (C_P) ($\mu\text{mol./l.}$)	Conc. of benzpyrene (C_{HC}) ($\mu\text{mol./l.}$)	Factor (f)	Mol. ratio (C_P^f/C_{HC})	Constant (C_P^f/C_{HC})
Caffeine	235.7	0.0155	1.045	15,200	1.94×10^4
	471.4	0.0317		14,900	1.95×10^4
	942.8	0.0618		15,230	2.05×10^4
	1,886	0.135		13,950	1.95×10^4
	2,357	0.179		13,150	4.11×10^4
	3,771	0.391		9,650	4.27×10^4
	4,714	0.608		7,760	4.05×10^4
	9,428	1.95		4,830	4.22×10^4
	11,780	2.90	1.74	4,055	4.19×10^4
	18,860	6.54		2,880	4.20×10^4
	21,650	8.64		2,510	4.05×10^4
	23,570	9.68		2,430	4.18×10^4
	28,280	13.15		2,155	4.25×10^4
	37,710	22.2		1,700	4.13×10^4
	47,140*†	32.6		1,450	4.15×10^4
	56,570*†	45.1		1,252	4.15×10^4
	58,920*	46.9		1,256	4.26×10^4
	113,140*	143.3		790	4.33×10^4
	226,270*	486		465	4.28×10^4
1:3:7:9-Tetramethyluric acid	223.1	0.0343	1.00	6,500	6.50×10^3
	356.9	0.0550		6,500	6.50×10^3
	446.2	0.0687		6,500	6.50×10^3
	892.4	0.135		6,600	6.64×10^3
	1,116	0.174		6,400	1.57×10^4
	1,785	0.409	1.78	4,360	1.56×10^4
	3,570	1.51		2,360	1.45×10^4
	4,462	2.11		2,120	1.52×10^4
	8,924	7.69		1,162	1.47×10^4
	17,850	26.7		669	1.46×10^4
	22,309	47.6		468	1.21×10^4
	26,770	54.2		494	1.48×10^4
	35,700*	114.0		324	1.18×10^4
	53,542*	189.7		283	1.46×10^4
	107,080*	709.5		151	1.34×10^4
	214,170*	2131		100.5	1.54×10^4
Theophylline	5,048*	0.198	1.74	25,450	1.40×10^7
	12,620*	0.96		13,180	1.39×10^7
	25,240*	3.13		8,080	1.44×10^7
	50,480*	11.1		4,540	1.37×10^7
	101,000*	35.7		2,830	1.41×10^7
1:3:7-Trimethyluric acid	3,808*	1.005	1.81	3,790	3.02×10^6
	4,760*	1.59		3,000	2.85×10^6
	9,519*	5.44		1,750	2.93×10^6
	19,040*	19.09		1,000	2.93×10^6
	23,800*	29.0		820	2.89×10^6
	38,075*	65.56		580	2.99×10^6

* Experiments carried out at 38°.

† Results identical at 20° and at 38°.

cluding hydrocarbons other than benzpyrene, f was found to be either near 1 or near 2, corresponding to a roughly linear or roughly quadratic relationship between the concentrations of hydrocarbon and purine. The actual values of f were usually found to be somewhat higher than 1 in the former case and somewhat lower than 2 in the latter. The values of k for any single curve, calculated from the empirical values of f , are on the whole in fairly good agreement.

The solvent effects on 3:4-benzpyrene of caffeine and of 1:3:7:9-tetramethyluric acid were investigated over an extensive range of concentrations and those of theophylline and 1:3:7-trimethyluric acid over a more limited range. The data are contained in Table 2. A measurable solvent effect is observed with as little as 0.005% caffeine or tetramethyluric acid. The break in the slope from the linear to the quadratic type of curve occurs at a caffeine concentration of 0.047%, and at an even lower concentration of tetramethyluric acid (0.02%), the latter substance having the higher solvent power. The benzpyrene concentrations at these points are identical, i.e. $0.16\mu\text{mol}$. The values of f found for the quadratic part of the curves are very similar for the four purines: 1.74 for caffeine and theophylline, 1.78 for tetramethyluric acid and 1.81 for trimethyluric acid. Obviously the range of the concentration curve is limited by the finite solubility of the purine concerned. But if we assume a higher solubility of the purine and a corresponding extension of the concentration curve, and if we further assume an unbroken continuation of the quadratic relationship between the two concentrations, a point will be reached where the concentration of dissolved benzpyrene may exceed that of the purine. As this is most unlikely, it cannot be expected that the quadratic type of curve will go on indefinitely; at high benzpyrene concentrations a return to the linear relationship is probable. Such a case has actually been observed, as will be shown in the following section.

It thus appears that for a similar concentration of dissolved benzpyrene a similar slope of the concentration curve is obtained, regardless of the purine employed as solubilizing agent.

The effect of purine concentration on the solubility of other hydrocarbons

The above rule was confirmed by the curves obtained with other hydrocarbons. Although great differences were found between the curves of different hydrocarbons, the curves obtained with different purines were practically identical over identical concentration ranges of one and the same hydrocarbon. An interesting case is that of pyrene (Table 3). Here the change in the slope of the concentration curve was in the reverse direction to that

observed with benzpyrene, i.e. from the quadratic to the linear type. As pointed out above, this behaviour is not surprising and would probably be of more general occurrence if the solubilities of hydrocarbons or purines were higher. The transition is again abrupt rather than gradual and occurs at a pyrene concentration of about $120\mu\text{mol}$, whether caffeine or tetramethyluric acid is used.

All the other hydrocarbons listed in Table 3 have uniform curves within the concentration ranges studied. Most of them are of the quadratic type although some are linear. Linear curves were observed with chrysene, 1:2:5:6-dibenzanthracene and coronene.

Temperature effects

Some of the experiments of Table 2 were carried out at 38° to avoid crystallization of the purine. Although the effect of temperature has not been studied in detail, some duplicate experiments were carried out simultaneously at 20° and at 38° . Identical results were obtained. It thus appears that the solvent power of a purine at a given concentration does not vary greatly with the temperature.

Comparison of the solvent power of purines

The solubilizing properties of a series of about 40 purines and purine derivatives were studied by measuring their effect on the solubility of 3:4-benzpyrene. For the purpose of comparison it would have been desirable to employ purine solutions at equal molar concentration. But as many purines are only slightly soluble in water, a very low concentration would have had to be chosen throughout. The resulting low concentration of dissolved benzpyrene would have increased the analytical difficulties and lowered the accuracy of the results. The parallelism of the concentration curves obtained with different purines makes it possible, however, to compare the solvent power of different purines at different molar concentrations by relating them to a common standard. The standard chosen was caffeine. The solvent power of caffeine, expressed by the molecular ratio, was plotted against the molar concentration of caffeine. Thus the molecular ratio could be obtained for any concentration of caffeine. If a molecular ratio had been determined with any purine, the molecular ratio for caffeine at the corresponding molar concentration was read from the graph and the following ratio calculated: molecular ratio of caffeine/molecular ratio of purine $\times 100$, giving the solvent power of the purine as a percentage of caffeine solvent power.

As far as their solubility would permit, purines were tested in 0.5% solution. Some difficultly soluble substances were tested at 38° . In a few

Table 3. *The influence of purine concentration on the solubility of various hydrocarbons*

Hydrocarbon	Purine	Conc. of purine (C_P) ($\mu\text{mol./l.}$)	Conc. of hydrocarbon (C_{HC}) ($\mu\text{mol./l.}$)	Factor (f)	Mol. ratio (C_P/C_{HC})	Constant (C_P^f/C_{HC})
Phenanthrene	Caffeine	9,428	37.95	1.83	248	4.81×10^5
		18,856	136.9		138	4.73×10^5
		37,712	478		79	4.81×10^5
		75,424	1,660		45.4	4.91×10^5
Anthracene	Caffeine	9,428	1.29	1.73	7,300	5.82×10^6
		18,856	4.35		4,330	5.74×10^6
		37,712	14.84		2,540	5.58×10^6
		75,424	42.3		1,780	6.48×10^6
Pyrene	Caffeine	9,428	29.9	1.98	315	2.48×10^6
		18,856	117.2		160.8	2.49×10^6
		37,712	274	1.26	137.5	2.08×10^6
		75,424	680		110.8	2.13×10^6
	T.M.U.	1,115	2.24	1.92	498	3.06×10^5
		2,231	8.65		258	2.99×10^5
		4,462	31.65		141	3.09×10^5
		8,924	125.5	0.96	71	2.93×10^5
		17,848	236		75.6	47.2
		35,695	460		77.6	48.8
		71,390	950		75.0	48.4
						45.5
Chrysene	Caffeine	9,428	7.1	1.0	1,328	1.33×10^3
		18,856	14.7		1,280	1.28×10^3
		37,712	29.3		1,290	1.29×10^3
		75,424	53.3		1,410	1.41×10^3
1:2-Benzanthracene	Caffeine	9,428	2.1	1.78	4,490	5.56×10^6
		18,856	8.1		2,330	4.95×10^6
		37,712	24.2		1,558	5.66×10^6
		75,424	84.7		889	5.56×10^6
9:10-Dimethyl-1:2-benzanthracene	Caffeine	9,428	0.308	1.95	30,600	1.91×10^8
		18,856	1.20		15,700	1.90×10^8
		37,712	4.60		8,200	1.92×10^8
		75,424	17.7		4,260	1.93×10^8
1:2:5:6-Dibenzanthracene	T.M.U.	8,924	0.43	1.09	20,700	4.58×10^4
		17,848	1.08		16,500	3.89×10^4
		35,695	2.34		15,800	3.81×10^4
		71,390	4.75		15,000	3.97×10^4
20-Methylcholanthrene	Caffeine	9,428	0.090	1.96	104,500	6.84×10^8
		18,856	0.346		54,500	6.92×10^8
		37,712	1.38		27,300	6.75×10^8
		75,424	5.52		13,620	6.60×10^8
	T.M.U.	8,924	0.138	1.98	64,600	4.97×10^8
		17,848	0.540		33,100	5.01×10^8
		35,695	2.16		16,500	4.95×10^8
		71,390	8.8		8,100	4.80×10^8
Coronene	Caffeine	9,428	0.61	1.0	15,500	1.55×10^4
		18,856	1.20		15,700	1.57×10^4
		37,712	2.12		17,800	1.78×10^4
		75,424	4.84		15,600	1.56×10^4
	T.M.U.	8,924	0.80	1.09	11,150	2.55×10^4
		17,848	1.71		10,450	2.55×10^4
		35,695	3.87		9,230	2.40×10^4
		71,390	7.86		9,060	2.52×10^4

T.M.U. = 1:3:7:9-tetramethyluric acid.

Table 4. *Solvent power of purines (towards 3:4-benzpyrene) relative to the solvent power of caffeine (= 100)*

	Concentration of		Molecular ratio (M.R.)	$\frac{\text{M.R.}_{\text{caffeine}}}{\text{M.R.}_{\text{purine}}} \times 100$
	Purine ($\mu\text{mol./l.}$)	Benzpyrene ($\mu\text{mol./l.}$)		
Hypoxanthine	14,700	0.16	92,000	3.7
*2-Methylhypoxanthine	33,300	0.452	73,600	2.6
Adenine	7,410	0.17	43,600	13.7
*2-Methyladenine	6,720	0.17	39,500	16.5
*2:9-Dimethyladenine	30,700	0.318	96,500	2.1
*2-Methylthioadenine	5,020	0.278	18,050	44.4
*2-Methylthio-9-methyladenine	5,180	0.468	11,060	71.5
Adenosine	17,000	0.337	50,400	5.95
Yeast adenylic acid	14,400	0.094	153,000	2.3
Guanine sulphate	3,310	0.179	18,550	55.5
*Guanosine	7,040	0.302	23,400	25.6
Yeast guanylic acid	27,600	1.25	22,100	9.8
*3-Methylxanthine	6,020	0.24	25,300	28.1
*7-Methylxanthine	12,040	0.37	32,700	12.2
*9-Methylxanthine	6,020	0.095	63,400	11.2
Theophylline (1:3-dimethylxanthine)	25,240	3.13	8,080	28.4
Paraxanthine (1:7-dimethylxanthine)	25,240	2.96	8,510	27
Theobromine (3:7-dimethylxanthine)	1,669	0.10	16,690	85
*Theophylline-7-d-galactopyranoside	14,620	0.107	137,000	2.5
8-Chlorotheobromine	3,108	0.246	12,620	85.5
Caffeine (1:3:7-trimethylxanthine)	23,570	9.68	2,430	100
8-Methoxycaffeine	9,630	4.03	2,390	199
8-Ethoxycaffeine	9,000	2.25	4,000	125
8-Chlorocaffeine	4,370	1.63	2,680	332
*1:3-Dimethyluric acid	9,350	0.84	11,150	44
*3:7-Dimethyluric acid	10,200	2.86	3,540	128
*3:9-Dimethyluric acid	5,100	0.30	17,000	47
*7:9-Dimethyluric acid	5,100	0.445	11,450	69
1:3:7-Trimethyluric acid	23,796	29.0	820	290
*1:3:9-Trimethyluric acid	14,300	2.83	5,050	69.3
*3:7:9-Trimethyluric acid	9,500	2.76	3,440	141
*1:7:9-Trimethyl-8-thiouric acid	22,150	0.083	266,000	0.9
1:3:7:9-Tetramethyluric acid	22,309	47.6	468	524
*1:3:7:9-Tetramethyl-8-thiouric acid	4,160	0.608	6,850	134
Caffeine methiodide	14,110	0.584	24,200	14.7
Caffeine methochloride	19,030	1.185	15,900	17.5
8-Hydroxy-9-methyl-8:9-dihydrocaffeine	41,000	0.47	87,400	1.8
*7:9-Dimethyldeoxyuric acid	27,820	0.377	74,000	3.0
1:3:7:9-Tetramethyldeoxyuric acid	24,050	1.30	18,500	12.7
3:7-Dimethyl-4:5-dihydroxydihydrouic acid	31,600	0	—	0
1:3:7-Trimethyl-4:5-dimethoxydihydrouic acid	73,500	0	—	0
Pilocarpine	43,270	0	—	0
[Sodium deoxycholate	5,110	1.10	4,650	173]
[Picric acid	10,910	0.305	35,850	12]

* At 38°.

cases substances tended to crystallize out during the experiment and the solutions had to be warmed up intermittently and before filtration. A number of purines, amongst them xanthine and uric acid, are so insoluble that they could not be examined. With xanthine, a 0.007 % solution could be obtained, but the solvent power at this concentration is so slight that the solubility of benzpyrene did not differ significantly from that in pure water. It is difficult to see how the positive result of Brock *et al.* (1938) was obtained.

The results are presented in Table 4. Though there are great differences in degree, there is not a

single purine which does not possess some solubilizing power, however slight. We are dealing therefore with a phenomenon which is common to, and characteristic of, the purines as a group. The differences in the constitution of the purines tested are, on the whole, of twofold nature: first, there are varying degrees of *N*-methylation; secondly, there may be present, at one or several of the carbon atoms in position 6, 2 or 8 of the purine ring electro-negative substituents, such as oxy-, alkoxy-, chloro-, thio-, alkylthio- or amino-groups. In general, solvent power increases with increasing *N*-methylation and with increasing number or strength

of electronegative substituents at the positions 6, 2 or 8. Hypoxanthine stands at one end of the scale and 1:3:7:9-tetramethyluric acid at the other.

N-methylation. The effect of *N*-methylation may be considered by comparing mono-, di- and trimethylxanthines, and also di-, tri- and tetramethyluric acids. On the whole, solvent power increases with the number of *N*-methyl groups, but clearly the four nitrogens of the purine ring are not equivalent in this respect. Thus, whereas 7- and 9-monomethylxanthines have about the same solvent power, both are much inferior to 3-methylxanthine. Amongst dimethylxanthines the 3:7-compound (theobromine) is more active than the 1:7- or the 1:3-compound both of which are of equal activity. The superiority of the 3:7-combination is confirmed in the series of dimethyluric acids; the other three dimethyluric acids tested all show considerably lower activities. Amongst them the 7:9-compound is more active than the 1:3- or 3:9-compound both of which are about equally active. Finally, 1:3:7-trimethyluric acid has about twice the solvent power of 3:7:9-trimethyluric acid and this again is about twice as active as 1:3:9-trimethyluric acid. Thus, whilst methylation of 1:3-dimethyluric acid in position 7, or of 3:7-dimethyluric acid in position 1, leads to a large increase in solvent power, methylation of 1:3- or 3:7-dimethyluric acids in position 9, or of 3:9-dimethyluric acid in position 1 has only a very small effect. These differences are probably connected with the different acidity of the *N*-bound H-atoms, which in the uric acid molecule decreases in the following order: 9 > 3 > 1 > 7 (Biltz, 1936).

A comparison of hypoxanthine with 2-methylhypoxanthine and of adenine with 2-methyladenine shows that methylation at a carbon atom is without effect. This is as might be expected, as will be discussed below.

Substitution by electronegative groups. The effect of the carbonyl group can best be judged by comparing xanthines with the corresponding uric acids, e.g. theophylline with 1:3-dimethyluric acid, theobromine with 3:7-dimethyluric acid, and caffeine with 1:3:7-trimethyluric acid. In all these cases the introduction of the carbonyl group at position 8 causes a significant increase in solvent power. Other electronegative substituents have a similar effect. Thus 8-chlorocaffeine is as active as 1:3:7-trimethyluric acid, whereas 8-methoxy- and 8-ethoxycaffeine are intermediate between 1:3:7-trimethyluric acid and caffeine in the order named. On the other hand, 8-chlorotheobromine is not more active than theobromine. The difference may be connected with the absence of a mobile H in the trimethylxanthine compounds.

The thio-group in position 8 is less efficient than the carbonyl group; this may be seen from the

activity of 1:3:7:9-tetramethyl-8-thiouric acid as compared with that of 1:3:7:9-tetramethyluric acid. On the other hand, the introduction of a methylthio-group into adenine or 9-methyladenine causes a large increase in solvent power.

From a comparison of hypoxanthine with adenine it appears that substitution by an amino-group has an even greater effect than that by a carbonyl group. Attention is drawn to the fact that aqueous solutions of methylated uric acids, saturated with benzpyrene, are non-fluorescent. This will be described fully in a subsequent paper (Weil-Malherbe, 1946).

Nucleosides and nucleotides. For an appraisal of the possible physiological significance of the solvent effect of purines it is of particular interest to determine whether it is shared by purine compounds such as nucleosides and nucleotides. Though attenuated the effect is still noticeable in both classes. In the nucleosides adenosine and guanosine it is reduced to 45% of that observed with the parent purine. On an absolute scale, however, the effect of guanosine especially is still quite considerable and is sufficient to be of physiological importance. Whereas in the nucleosides ribose is attached to position 9 (Gulland & Holiday, 1936), the introduction of a galactose residue at position 7, as in theophylline-7-*d*-galactopyranoside, leads to a reduction in solvent power of about 90%.

Related compounds. A number of compounds was prepared in which, though the purine skeleton is still intact, it has undergone slight modifications. In all these substances the solvent effect was either greatly reduced or completely abolished.

Caffeine methiodide and methochloride (I) on treatment with alkali give the pseudo-base 8-hydroxy-9-methyl-8:9-dihydrocaffeine (II) or 'caffeine methohydroxide'. Formula (II), which was proposed for the pseudobase by Meyer & Jacobson (1923), is in accordance with that accepted for analogous heterocyclic compounds. Further support is provided by the fact that 1:3:7:9-tetramethyluric acid is formed from the pseudobase on oxidation with hypiodite.

0.5 g. of the pseudobase was dissolved in 5 ml. 2*N*-NaOH and 5 ml. *N*-I₂ solution were added. After 15 min. at room temperature the solution was acidified with conc. HCl, excess I₂ was removed by a few drops of dilute NaHSO₃ solution and the mixture was extracted 5 times with 10 ml. chloroform. The chloroform extract was dried and evaporated. The residue was recrystallized from methanol, yielding 82 mg. needles, m.p. 225°. The mixed melting-point with authentic tetramethyluric acid gave no depression.

In the pseudobase (II) the double bond adjacent to position 8 has disappeared. The substance no longer contains the iminazole nucleus characteristic

of a purine and accordingly has only a very slight solvent power.

The constitution of the deoxyuric acids is still an open question, as the formula proposed by Biltz (1921) is incompatible with the current conception of nitrogen valency; moreover, if Biltz's formula, or some modification of it, were correct, one might expect the salts of 1:3:7:9-tetramethyldeoxyuric acid to be identical with the corresponding quaternary caffeine salts. This is, however, not the case. Whatever their constitution, these compounds have also a greatly reduced solvent power compared with the corresponding uric acids. Finally, the fact that the uric acid 'glycols' which have lost the 4:5-double bond (e.g. III) have at the same time completely lost every trace of solvent power suggests

that the iminazole nucleus present in the purine molecule is essential for solubilizing properties.

Pilocarpine which, according to J. Keilin (1943), has a solubilizing effect on porphyrins is inactive towards benzpyrene. The last two entries in Table 4 show (1) that the solvent effect of purines is of the same order of magnitude as that of sodium deoxycholate; there are even some purines which are much better solubilizers than this bile salt. (2) That picric acid which forms molecular compounds with hydrocarbons has also a slight but definite solvent action on benzpyrene.

The effect of alkali

The solvent power of all those purines which possess a mobile H-atom and therefore an acidic function is greatly weakened, and in many cases practically lost, in alkaline solution (Table 5). The position of the acidic function in the purine molecule is immaterial in this respect. Pure barium 1:3:7-trimethylurate, dissolved in water, shows the same inhibition of solvent power as 1:3:7-trimethyluric acid dissolved in 0.1N-NaOH. From this it can be concluded that the ionization of the acidic group in the purine is responsible for the loss of solvent action, and not the alkaline reaction due to the presence of excess alkali.

There are no mobile H-atoms in either caffeine or 1:3:7:9-tetramethyluric acid, and in consequence the solvent power of these substances is not seriously affected in alkaline solution. The small reduction observed is almost certainly due to the fact that both compounds are very sensitive to alkali and are broken down to some extent during the experiment. Caffeine is a weak base and forms salts with mineral acids. Its solvent power is, however, unaffected in acid solution.

Table 5. *The influence of alkali or acid on solubilizing activity*

	Purine conc. ($\mu\text{mol.}$)	Benzpyrene concentration ($\mu\text{mol.}$)	Molecular ratio (M.R.)	$\frac{\text{M.R.}_{\text{caffeine}}}{\text{M.R.}_{\text{purine}}} \times 100$
Xanthine in 0.1N-NaOH	29,400	0.33	88,000	2.36
Guanine in 0.1N-NaOH	16,520	0.26	64,000	4.8
Theobromine in 0.1N-NaOH	27,800	0.33	84,500	2.6
Theophylline in 0.1N-NaOH	25,240	0.10	252,400	0.9
1:3-Dimethyluric acid in 0.1N-NaOH	23,350	0.10	233,500	1.03
3:7-Dimethyluric acid in 0.1N-NaOH	25,500	0.10	255,000	0.9
1:3:7-Trimethyluric acid in 0.1N-NaOH	23,800	1.0	23,800	10.0
Barium 1:3:7-trimethylurate in water	15,500*	0.45	34,400*	9.5
1:3:7-Trimethyluric acid in 0.1N-NaOH	23,800	0.050	474,000	0.5
3:7:9-Trimethyluric acid in 0.1N-NaOH	23,800	0.206	115,500	2.03
Caffeine in 0.1N-H ₂ SO ₄	23,570	9.68	2,430	100.0
Caffeine hydrochloride in water	21,650	8.64	2,500	100.0
Caffeine in 0.1N-NaOH	23,570	6.35	3,700	65.4
1:3:7:9-Tetramethyluric acid in 0.1N-H ₂ SO ₄	22,309	50.4	443	554
1:3:7:9-Tetramethyluric acid in 0.1N-NaOH	22,309	31.0	719	341

* Calc. for 1 equiv. of trimethyluric acid.

Comparison of the solubility of polycyclic aromatic hydrocarbons in purine solutions

In Table 6 the molecular ratios obtained with various hydrocarbons in 0.5% caffeine and 0.5% tetramethyluric acid solutions are shown. It appears (1) that the solubility decreases with the number of condensed rings, (2) that with an equal number of rings substances with an angular arrangement of rings are more soluble than those with a straight arrangement (compare, for example, phenanthrene and anthracene or pyrene and naphthacene), and (3) that the aliphatic side chains in 9:10-dimethyl-1:2-benzanthracene and 20-methylcholanthrene greatly reduce the solubility in comparison with the parent hydrocarbon, 1:2-benzanthracene. 9:10-Dimethylantracene, on the other hand, has a solubility not very different from anthracene. It seems as if steric hindrance may operate in the case of the 1:2-benzanthracene derivatives.

For every hydrocarbon tested, except chrysene, tetramethyluric acid is a more efficient solvent than caffeine, though in varying degree. This superiority is particularly marked in the case of 3:4-benzpyrene.

Table 6. *Comparative solubility of hydrocarbons in purine solutions*

	Molecular ratio		$\frac{\text{M.R.}_{\text{caff.}}}{\text{M.R.}_{\text{T.M.U.}}} \times 100$
	In 0.5% caffeine	In 0.5% T.M.U.	
Phenanthrene	114	45.5	250
Anthracene	3,680	950	390
9:10-Dimethylantracene	2,680	1,166	230
Pyrene	151	75	200
Chrysene	1,280	1,385	93
1:2-Benzanthracene	1,960	617	320
9:10-Dimethyl-1:2-benzanthracene	12,100	3,880	310
20-Methylcholanthrene	44,600	25,800	170
3:4-Benzpyrene	2,430	468	520
1:2:5:6-Debenzanthracene	18,800	16,000	120
Coronene	15,700	10,100	150
Naphthacene	Practically insoluble		
Rubrene			

T.M.U. = 1:3:7:9-Tetramethyluric acid.

The formation of molecular compounds

The solubilizing properties of sodium deoxycholate have frequently been linked with the tendency of deoxycholic acid to form molecular compounds. The search for molecular compounds between purines and polycyclic aromatic hydrocarbons therefore at once suggested itself. The existence of such compounds could indeed be shown.

The purine chosen for these experiments was 1:3:7:9-tetramethyluric acid. Well-defined molecular compounds were formed with pyrene, 3:4-benzpyrene and coronene. Both the last two compounds contain the pyrene ring system and may therefore be regarded as derivatives of pyrene. No molecular compounds were obtained with the following hydrocarbons: phenanthrene, anthracene, chrysene, 20-methylcholanthrene and 1:2:5:6-dibenzanthracene. The unchanged components were deposited on cooling the hot benzene solutions containing hydrocarbon and tetramethyluric acid.

The molecular compound formed between pyrene and tetramethyluric acid contains 1 mol. of tetramethyluric acid per molecule of pyrene, whereas the compounds formed with 3:4-benzpyrene and coronene both contain 2 mol. of tetramethyluric acid per molecule of hydrocarbon. The description of the preparation of these molecular compounds follows.

Pyrene-tetramethyluric acid. 2 g. pyrene were dissolved in 30 ml. benzene. The solution was refluxed over tetramethyluric acid for about 20 min. and filtered hot. On cooling, the molecular compound crystallized in the form of colourless needles. M.p. 207–208°. (Found (Weiler): C, 70.4; H, 5.2; N, 13.0. $\text{C}_{16}\text{H}_{10}\cdot\text{C}_4\text{H}_4\text{O}_3\text{N}_4$ requires C, 70.4; H, 5.2; N, 13.2%.)

Benzpyrene-tetramethyluric acid. 2 g. benzpyrene and 1 g. tetramethyluric acid were dissolved in 80 ml. hot benzene. On cooling, unchanged tetramethyluric acid was filtered off and the filtrate concentrated to about 30 ml. On cooling, the molecular compound was deposited in the form of pale yellow needles. M.p. 192°. Fluorescence analysis after solution in benzene, extraction with water and chromatography: 0.259 mg. contained 0.093 mg. benzpyrene. (Found: 35.9% benzpyrene; calc. for 1 benzpyrene: 2 tetramethyluric acid: 36.0% benzpyrene. Analysis (Weiler): Found: C, 65.1; H, 5.2; N, 15.7. $\text{C}_{20}\text{H}_{12}\cdot 2\text{C}_4\text{H}_4\text{O}_3\text{N}_4$ requires C, 65.1; H, 5.2; N, 16.0%.)

Coronene-tetramethyluric acid. 200 mg. coronene were dissolved in about 50 ml. hot xylene and 600 mg. tetramethyluric acid in another 50 ml. hot xylene. The clear solutions were mixed while hot. Pale yellow needles appeared on cooling and were recrystallized from xylene. The compound started sintering at about 260°, but did not melt below 340°. (Found (Weiler): C, 68.0; H, 4.7; N, 14.9. $\text{C}_{24}\text{H}_{12}\cdot 2\text{C}_4\text{H}_4\text{O}_3\text{N}_4$ requires C, 67.4; H, 4.9; N, 15.0%.)

It is noteworthy that these molecular compounds show no change or intensification of colour as compared with the parent hydrocarbon. This is in contrast to the highly coloured adducts of the quinhydrone type or the molecular compounds between nitro-compounds and hydrocarbons, phenols or amines, or between carbonyl-compounds and metal salts. Such compounds are probably linked together by an electro-valency bond and are of ionic character (Weiss, 1942). The molecular compounds between tetramethyluric acid and hydrocarbons,

however, resemble the colourless adducts of deoxycholic acid which are assumed to be linked together by co-ordinate bonds. Fieser & Newman (1935) showed that uniform crystalline compounds of hydrocarbon-choleic acids could be obtained in some cases, but not in others, and that they conformed to the co-ordination principle inasmuch as they contained per molecule of hydrocarbon 2, 3, or 4 mol. of deoxycholic acid, the number increasing with the molecular size of the hydrocarbon.

The formation of mixed crystals

When a hot concentrated aqueous solution of caffeine or tetramethyluric acid was saturated with pyrene or benzpyrene and filtered, the crystals which appeared on cooling contained a certain proportion of the hydrocarbon. Thus the tetramethyluric acid crystals contained 1 mol. of benzpyrene for 40.6 mol. of tetramethyluric acid, and 1 mol. of pyrene for 27.3 mol. of tetramethyluric acid. In contrast to the molecular compounds the crystals were indistinguishable in melting-point and appearance from the pure purine. But on microscopic examination in filtered ultraviolet light the crystals showed a vivid, uniform fluorescence. Recrystallization from water or better from ethanol led to a progressive diminution in hydrocarbon content.

DISCUSSION

The mechanism of solubilization

Purines differ from many other solubilizing agents either by the fact that they are active in very low concentrations or by the fact that they do not form colloidal solutions. Of the various types of solubilizing mechanisms discussed by McBain (1942) only the 'choleic acid principle' seems to fit the case of the purines. The choleic acid principle assumes a causal connexion between the solubilizing action of bile salts and the formation of chemical co-ordination compounds of choleic acids. Against this mechanism the objection has been raised that molecular co-ordination compounds are largely dissociated in solution as shown by spectroscopic evidence (v. Halban & Zimpelmann (1925) and Jones & Neuworth (1944) for picrates and related compounds; Winterstein & Vetter (1934) and Lorenz (1935) for choleic acid compounds) and by chemical evidence (Sobotka & Kahn, 1932; Marx & Sobotka, 1936).

Even though molecular compounds be largely dissociated in solution, this does not exclude the possibility that the forces responsible for the formation of molecular compounds may bring about solubilization. Though largely dissociated, there will always be a percentage of undissociated molecular compound present in solution, however small. In kinetic terms this means that the mole-

cular compound has a very short lifetime in solution. But even after dissociation some interaction may continue in attenuated form sufficient to account for solubilization. The existence of molecular compounds between purines and hydrocarbons is a further strong argument for the identity of the forces responsible for solubilization and molecular compound formation. Those hydrocarbons, pyrene, benzpyrene and coronene, which were shown to form molecular compounds, are also more soluble than other hydrocarbons of similar molecular size, which do not form molecular complexes and where therefore the interacting forces are less strong. Finally, it has been shown that picric acid, a complex former *par excellence*, also has a solubilizing effect.

The fact that there exists either a linear or a quadratic relationship between the concentrations of hydrocarbon and purine can easily be interpreted in terms of molecular interaction. If we assume that solubilization is due to interaction of 1 mol. purine with 1 mol. hydrocarbon, then, according to the Law of Mass Action:

$$C_P \times C_{HC} = kC_{HC.P},$$

where C_P = concentration of purine, C_{HC} = concentration of hydrocarbon and $C_{HC.P}$ = concentration of solubilized hydrocarbon. Since C_{HC} is present as solid phase, it does not enter into the equation which becomes

$$C_P = kC_{HC.P},$$

i.e. the concentration of dissolved hydrocarbon is proportional to the concentration of purine.

Similarly for the case in which 1 mol. hydrocarbon reacts with 2 mol. purine:

$$(C_P)^2 = kC_{HC.2P},$$

i.e. the concentration of dissolved hydrocarbon is proportional to the square of the purine concentration.

It is interesting to note that benzpyrene which has a quadratic type of concentration curve at high purine concentrations forms a molecular compound with 2 mol. tetramethyluric acid, and pyrene, with a linear concentration curve at high purine concentrations, forms a molecular compound with 1 mol. The analogy breaks down in the case of coronene which has a linear concentration curve, but forms a molecular compound with 2 mol. tetramethyluric acid, but as the type of concentration curve is variable with the conditions such a disagreement is not particularly surprising.

*The influence of constitution on the solubilizing effect**

The examination of a large number of purines and purine derivatives has shown that the solubilizing

* This section is based on discussions with Dr J. Weiss.

effect increases (1) with the number and position of *N*-methyl groups, (2) with the number and strength of electronegative substituents at positions 6, 2 and 8 of the purine ring. It is not intended, and on the basis of the available facts hardly possible, to attempt a detailed interpretation of constitutional influences; but a qualitative picture may be visualized in broad outlines.

A solubilizing agent must be able to interact with both the solvent and the insoluble substance. If the solvent is water, it must have hydrophilic and hydrophobic properties. In the particular case under discussion solubilization implies an interaction between hydrocarbon and purine molecules on the one hand and between purine and water molecules on the other. The (van der Waals) forces between hydrocarbon and purine largely depend on the polarizability of the interacting molecules. Like molecular refraction to which it is closely related polarizability is of an approximately additive character. The polarizability of aromatic hydrocarbons is due to their system of conjugated double bonds. As for the purine, its polarizability will depend on the number, size and nature of substituents and on the number and position of double bonds. The introduction of either *N*-methyl groups or of electronegative groups will not only increase the polarizability of the molecule, but, owing to the considerable dipole moment of these groups, also its hydrophilic properties. Methylation of a C-atom, on the other hand, will, though also increasing the polarizability of the molecule, decrease its hydrophilic character. As has been shown, the solubilizing effect is only increased by substitutions of the former kind.

Whereas a soap molecule owes its hydrophilic properties, and thereby its activity as a solubilizing agent, to its electric charge, ionization of a purine molecule greatly reduces its solubilizing properties. In the purine molecule with its more or less conjugated character an electric charge cannot be strongly localized, as the hydrophilic and hydrophobic groups are not geometrically isolated. A charge on the purine molecule will therefore greatly reinforce the attraction of the water dipoles to the detriment of the forces interacting with the hydrocarbon. The great increase in water-solubility of many ionized purines, especially uric acids, is an expression of this change, just as, on the other hand, ionization reduces, and in many cases practically abolishes, the solvent power for hydrocarbons. It does not matter in this respect whether ionization is of the type encountered in urates where a negative charge resides at one or two, enolized carbonyl groups, or whether, as in caffeine methiodide, N_9 carries a positive charge. It might be expected that caffeine ions which also have a positive charge at N_9 should show the same reduc-

tion of solvent power, but as caffeine is a very weak base, their concentration is insignificant and cannot affect the solubilizing power of the undissociated caffeine even in strongly acid solution.

It may be concluded that hydrophilic properties are favourable to the solubilizing effect of a purine up to a certain degree, but when they become too strong, they are highly unfavourable. Solubilization may therefore be conceived as a competition between water molecules and the molecules of the insoluble substance for the molecules of the solubilizing agent, in which a proper balance must be maintained with neither side outweighing the other. In some solubilizers, such as fatty acids or bile acids, this balance is established only after ionization; others, like purines, possess it in the undissociated state and ionization leads to such an increase of water-attracting forces that hydrocarbon molecules are 'crowded out' and have no longer the chance to react with the purine molecules.

The inactivity of acidic purines in the ionized state is the most probable explanation of J. Keilin's results. Of the purines tested by her only caffeine and 8-chlorocaffeine were non-acidic and these were the only active substances in her experiments, which were carried out either in alkaline solution or in acetate buffer of pH 4.5. As most purines are relatively strong acids, they may already be partly ionized at this pH. It would be interesting to study the solvent effect of purines on porphyrins in unbuffered solutions.

The physiological significance of the solubilizing effect of purines

Though it is tempting to speculate on the physiological significance of the effects described two facts must be borne in mind: (1) Despite their comparably high activity at relatively low concentrations it is unlikely that purines, and especially the less active unmethylated purines which alone occur in animal tissues, are ever present in intracellular concentrations high enough to display any considerable solubilizing activities. (2) The unmethylated purines of animal origin are all acidic and are therefore likely to be ionized in the buffered medium of the cell, and it has been shown that in this state they are inactive. On the other hand, it is difficult to believe that this remarkable property of the purines is quite unconnected with their biological function. The solubilizing power of the bile salts certainly has a physiological function in the process of digestion and resorption. But the great toxicity of these highly surface-active compounds limits their function to extracellular activity. Might they not have their non-toxic, intracellular counterpart in the form of some purine compound, possibly nucleoproteins? In particular the special affinity between hydrocarbons and purines suggests a

similar affinity between hydrocarbons and nucleoproteins. Such a reaction may be connected with carcinogenesis which is largely a problem of nuclear metabolism. Leiter & Shear (1943) found a more or less pronounced retardation or reduction of tumour formation induced by subcutaneous injections of benzpyrene when hypoxanthine, xanthine, guanine, caffeine or nucleic acid was added to the lipid solvent. This protective action, if confirmed, might be explained by the competition of these substances with the cellular nucleoprotein for the carcinogen.

The possible significance of the solubilizing effect for the pharmacological activity of methylated purines or for the metabolism of the plants which synthesize them can merely be touched upon. In this connexion it is worth mentioning that 1:3:7:9-tetramethyluric acid, the most active solubilizer of the purine family, and one which, like caffeine, is not subject to ionization, has been shown to occur naturally in tea leaves (Johnson, 1937).

There are indications that other organic nitrogen compounds possess solubilizing properties of the type described for the purines. According to Brock *et al.* (1938) antipyrine and urethane are slightly, but definitely active towards benzpyrene (see also Herrmann, Sulzberger & Baer, 1942). It may be added that Brock *et al.* found sols of albumins and globulins inactive. As serum is sometimes used as a solvent for carcinogenic hydrocarbons, it seems likely that the active agent of serum is not its protein, but its lipid fraction.

SUMMARY

A quantitative study has been made of the solvent effect of purines on 3:4-benzpyrene and other polycyclic aromatic hydrocarbons and the following results were obtained:

(1) There exists either a roughly linear or a roughly quadratic relationship between the concentrations of hydrocarbon and purine in solution. The exponential coefficients expressing this relationship were usually found to be somewhat >1 in the former case and somewhat <2 in the latter. While the quadratic type is more frequent, the linear type was observed with chrysene, 1:2:5:6-dibenzanthracene and coronene. With 3:4-benzpyrene a sudden change from the linear to the quadratic type occurs at low concentrations and with pyrene one in the opposite direction at high concentrations.

(2) A noticeable solvent effect is observed with as little as 0.005% caffeine or tetramethyluric acid solutions.

(3) For similar concentrations of dissolved hydro-

carbon the curves showing the increase of hydrocarbon concentration with purine concentration have similar slopes, regardless of the purine employed as solubilizing agent.

(4) Solubilizing power is a property common to, and characteristic of, the purines as a group. It increases (1) with increasing N-methylation, the four nitrogens of the purine ring not being equivalent in this respect (2) with increasing number and strength of electronegative substituents in the positions 6, 2 and 8.

(5) Though attenuated, the solvent effect is shown by nucleosides and nucleotides.

(6) Slight modifications of the purine ring, such as quaternization of N_9 or the loss of the 4-5 double bond or of the double bond adjacent to C_8 lead to great reduction or complete loss of solvent power.

(7) Ionization of purines with acidic functions wherever situated greatly inhibits the solvent effect and in most cases practically abolishes it.

(8) The solubility of polycyclic aromatic hydrocarbons in purine solutions decreases with the number of condensed rings. Of substances with an equal number of rings those with an angular arrangement of rings are more soluble than those with a straight arrangement.

(9) Pyrene, 3:4-benzpyrene and coronene form well-defined molecular compounds with 1:3:7:9-tetramethyluric acid. The components are present in the proportion of 1:1 in the pyrene compound, and of 1 hydrocarbon:2 purines in the other two compounds. These molecular compounds show no change or intensification of colour as compared with the parent hydrocarbon.

(10) The results are discussed and the following conclusions are drawn: (1) Solubilization is brought about by a chemical interaction between a hydrocarbon molecule and one or two purine molecules. The forces underlying this affinity are probably identical with those which are responsible for the formation of molecular compounds though possibly existing in attenuated form. (2) The constitutional factors which favour the solvent power tend to increase both the polarizability and the hydrophilic character of the molecule. It is emphasized that a proper balance between these two factors must be maintained and that an increase of hydrophilic character beyond a certain degree, such as is entailed by the acquisition of an electric charge, has the opposite effect.

I am greatly indebted to Prof. E. L. Kennaway, F.R.S., for a gift of 3:4-benzpyrene, to Prof. A. R. Todd, F.R.S., for various purines, and to Miss J. Keilin for a specimen of paraxanthine.

REFERENCES

- Biltz, H. (1921). *Liebigs Ann.* **426**, 237.
 Biltz, H. (1936). *J. prakt. Chem.* **145**, 65.
 Biltz, H. & Bülow, H. (1921a). *Liebigs Ann.* **426**, 246.
 Biltz, H. & Bülow, H. (1921b). *Liebigs Ann.* **426**, 283.
 Biltz, H. & Damm, P. (1914). *Liebigs Ann.* **406**, 22.
 Biltz, H. & Heidrich, D. (1921). *Liebigs Ann.* **426**, 290.
 Biltz, H. & Heyn, M. (1916). *Liebigs Ann.* **413**, 180.
 Biltz, H. & Heyn, M. (1921). *Liebigs Ann.* **423**, 185.
 Biltz, H. & Krzikalla, H. (1921). *Liebigs Ann.* **423**, 259.
 Biltz, H. & Krzikalla, H. (1927). *Liebigs Ann.* **457**, 131.
 Biltz, H. & Pardon, H. (1930). *Ber. dtsh. chem. Ges.* **63**, 2876.
 Biltz, H. & Strufe, K. (1916). *Liebigs Ann.* **413**, 197.
 Biltz, H. & Topp, E. (1911). *Ber. dtsh. chem. Ges.* **44**, 1524.
 Brock, N., Druckrey, H. & Hamperl, H. (1938). *Arch. exp. Path. Pharmac.* **189**, 709.
 Fieser, L. F. & Newman, M. S. (1935). *J. Amer. chem. Soc.* **57**, 1602.
 Fischer, E. (1882). *Liebigs Ann.* **215**, 253.
 Fischer, E. (1884). *Ber. dtsh. chem. Ges.* **17**, 1785.
 Fischer, E. (1895). *Ber. dtsh. chem. Ges.* **28**, 2482.
 Fischer, E. & Reese, L. (1883). *Liebigs Ann.* **221**, 336.
 Gulland, J. M. & Holiday, E. R. (1936). *J. chem. Soc.* p. 765.
 v. Halban, H. & Zimpelmann, E. (1925). *Z. phys. Chem.* **117**, 461.
 Herrmann, F., Sulzberger, M. B. & Baer, R. L. (1942). *Science*, **96**, 451.
 Johnson, T. B. (1937). *J. Amer. chem. Soc.* **59**, 1261.
 Jones, R. C. & Neuworth, M. B. (1944). *J. Amer. chem. Soc.* **66**, 1497.
 Keilin, J. (1943). *Biochem. J.* **37**, 281.
 Leiter, J. & Shear, M. J. (1943). *J. nat. Cancer Inst.* **3**, 455.
 Lorenz, E. (1935). Quoted by Fieser & Newman (1935).
 McBain, J. W. (1942). *Advances in Colloid Science*, **1**, 99. New York: Interscience Publishers, Inc.
 Marx, W. & Sobotka, H. (1936). *J. organ. Chem.* **1**, 275.
 Meyer, V. & Jacobson, P. (1923). *Lehrbuch der organischen Chemie*, 1st and 2nd ed. Berlin and Leipzig: Walter De Gruyter and Co.
 Schmidt, E. & Schilling, E. (1885). *Liebigs Ann.* **228**, 141.
 Sobotka, H. & Kahn, J. (1932). *Ber. dtsh. chem. Ges.* **65**, 227.
 Weil-Malherbe, H. (1944). *Biochem. J.* **38**, 135.
 Weil-Malherbe, H. (1946). *Biochem. J.* **40**, 363.
 Weiss, J. (1942). *J. chem. Soc.* p. 245.
 Winterstein, A. & Vetter, H. (1934). *Hoppe-Seyl. Z.* **240**, 169.

Effects of Purines on Fluorescent Solutions

By H. WEIL-MALHERBE, *Cancer Research Laboratory, North of England Council of the British Empire Cancer Campaign, Royal Victoria Infirmary, Newcastle upon Tyne*

(Received 1 January 1946)

The starting-point of the present investigation was the observation that the fluorescence of an aqueous benzpyrene-caffeine solution disappears on acidification and reappears on neutralization. This led to a systematic examination of the effects on fluorescence of a large number of purines and purine derivatives. Not only the fluorescence of 3:4-benzpyrene, but also that of other hydrocarbons and non-hydrocarbons was studied.

METHODS

Fluorescence measurements were carried out with a direct-reading fluorimeter (Weil-Malherbe, 1944b). Unless otherwise stated, measurements were done in air, since the quenching effect of oxygen is not very marked in aqueous solvents owing to the slight solubility of the gas in water. Moreover, preliminary experiments had shown that the quenching effect of acid aqueous caffeine solutions is the same in air and in pure nitrogen.

RESULTS

The effect of acid caffeine solutions

If H_2SO_4 is added in increasing concentrations to an aqueous caffeine solution of 3:4-benzpyrene, the

fluorescence diminishes progressively and disappears completely at a 0.2N-concentration of acid (Table 1). Other acids have a similar effect, depending on their degree of dissociation. On neutralization the fluorescence is almost completely restored.

Table 1. *Reversible quenching of the fluorescence of 3:4-benzpyrene (c. 1 $\mu\text{g./ml.}$) in 1% aqueous caffeine solution by acids*

Final concentration of acid added	Fluorescence as % of fluorescence in aqueous caffeine solution	
	With acid	After neutralization with N-NaOH
0.001N- H_2SO_4	86.6	—
0.002N- H_2SO_4	66.7	—
0.01N- H_2SO_4	36.1	—
0.02N- H_2SO_4	13.5	94.4
0.2N- H_2SO_4	0	91.3
0.02N HCl	11.6	96.6
2 vol % H_3PO_4 (sp.gr. 1.75)	2.1	92.7
0.4N acetic acid	57.3	96.5
2% citric acid	28.0	98.2

The first question to be decided was, whether the H-ions as such or the caffeine ions formed on