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Further Investigations on the Solubilization of Carcinogenic Hydrocarbons by Association Colloids

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In a previous paper[1] it has been shown that the fat-soluble, but water-insoluble, polycyclic hydrocarbons with carcinogenic properties can be brought into aqueous solutions with the aid of association colloids. These solutions are homogeneous and stable. The investigation has been continued on different lines. The previously published solubility values have been verified and the investigation has been extended to include several new association colloids and some new polycyclic hydrocarbons. The properties of the prepared aqueous solutions of these substances have been investigated. The ability of these solutions to penetrate in the skin and other tissues and to carry the carcinogen with them, as well as the carcinogenic activity of the hydrocarbons in these solutions have been studied.

SOLUBILIZATION STUDIES

We have determined the solubilities of five hydrocarbons: 1,2-benzanthracene (BA), 9,10-dimethyl-1,2-benzanthracene (DMBA), 1,2,5,6-dibenzanthracene (DBA), 20-methylchloantrene (MC), and 3,4-benzpyrene (BP), in aqueous solutions of the following association colloids: potassium myristate, sodium oleate, sodium lauryl and sodium myristyl sulphate, sodium cholate, sodium deoxycholate, sodium taurocholate, a cationic association colloid mixture ("Quatrogan"), the alkyl aryl polyether alcohol "Triton N 100", and the polyoxyethylene sorbitan monopalmitate "Tween 40". In addition, we have found that solutions of a number of commercial products, *viz.* "Aerosol IB", "Aerosol AY", "Aerosol MA", "Aerosol OT", "Cetavlon", "Nacconol NR", "Tween 20", "Tween 60", "Tween 80", "Atlas G 2149", "Atlas G 2152", "Atlas G 2153", and "Triton WR 1339", are able to solubilize polycyclic hydrocarbons.

The 3,4-benzpyrene was a product of Hoffmann-La Roche & Co, Basel, while the other hydrocarbons were from Eastman-Kodak Co, Rochester, N. Y. Potassium myristate, sodium oleate, sodium cholate and sodium deoxycholate were prepared from the corresponding acids by dissolving the latter in absolute ethanol and adding an equivalent amount of sodium or potassium alcoholate to the warm solutions. The acids were of the following qualities: myristic acid: Eastman-Kodak Co, Rochester, N. Y.; oleic acid: May & Baker Ltd, Dagenham; cholic acid: Hoffmann-La Roche & Co, Basel (recrystallized from ethanol); deoxycholic acid: Hoffmann-La Roche & Co, Basel (recrystallized from acetic acid). Sodium taurocholate was obtained by purification of a product of Merck, Darmstadt. Sodium lauryl sulphate and sodium myristyl sulphate were prepared from the commercial products "Duponol PC" and "Duponol ME" (Du Pont de Nemours Wilmington, Del.), respectively, by ethanol extraction and repeated recrystallization from ethanol. They may have contained slight amounts of homologues. All these substances were dried in vacuo over phosphorous pentoxide before use. "Quatrogan" (Ab. Recip, Stockholm) was used as the commercial product containing 10 per cent cetyl pyridinium chloride and 90 per cent alkyl dimethyl benzyl ammonium chlorides with alkyl groups containing from 8 to 18 carbon atoms. "Triton N 100" was the product of Rohm & Haas Company, Philadelphia, Pa. It consists of an alkyl aryl polyether alcohol with the average molecular weight 710. Presumably the preparation is a mixture of different homologues. "Triton NE", which we have used earlier, is a 32 per cent aqueous solution of "Triton N 100". "Tween 40" was the commercial product of Atlas Powder Company, Wilmington, Del. It consists of polyoxyethylene sorbitan monopalmitate.

As all the hydrocarbons studied are strongly fluorescent in solution their solubilities in the association colloid solutions were determined by measuring the fluorescence intensities. The procedure was the following: The colloid solution was saturated with the hydrocarbon by shaking it with an excess of the latter in a ground-glass stoppered test-tube in a thermostat at 40° C. When equilibrium was reached after 40 to 70 hours, the excess of hydrocarbon was removed by filtration. The intensity of the fluorescence of the saturated solution was compared with that of a solution of the same colloid concentration containing a known amount of the hydrocarbon in question. The latter solution was prepared in the following manner: A definite volume (0.01—0.18 ml) of a standard solution of the hydrocarbon in benzene was measured into a test-tube by means of an "Agla" micrometer syringe. The benzene was then evaporated in vacuo at room temperature in the dark. A known amount of the colloid solution was added and the hydrocarbon was brought into solution by shaking under the same conditions as in the preparation of the saturated solution. It was necessary to adjust the hydrocarbon concentration of both solutions to within the range in which a linear relation exists between the fluorescence intensity and the hydrocarbon concentration. In many cases the saturated solutions were diluted with the original colloid solution before measurement. The concentrations of both solutions were adjusted to be relatively near each other. As in our previous work, the intensity of the fluorescence

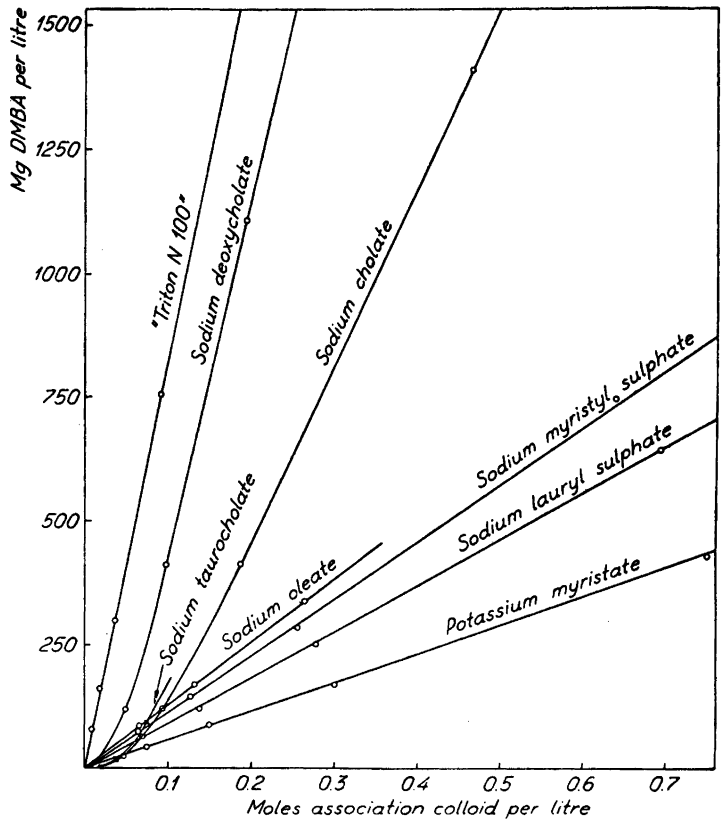

Fig. 1. The solubility of 9,10-dimethyl-1,2-benzanthracene in various association colloid solutions.

was measured with a Pulfrich Step-photometer provided with an ultraviolet mercury lamp and with a thermostat arrangement which made measurements possible at 40° C.*

The solubility values obtained in this manner are given in Table 1 and Figs. 1—5.

The previously published solubility values [1] have been confirmed on the whole. These reveal again that the solubilities of the hydrocarbons increase

* Quite recently H. B. Klevens (*J. Phys. & Colloid Chem.* **54** (1950) 283) has reported on the solubilities of a number of polycyclic hydrocarbons, some carcinogenic (methylcholantrene, 1,2,5,6-dibenzanthracene) in potassium laurate solutions at 25° C. He determined the solubilities by measuring the absorption in the ultra violet.

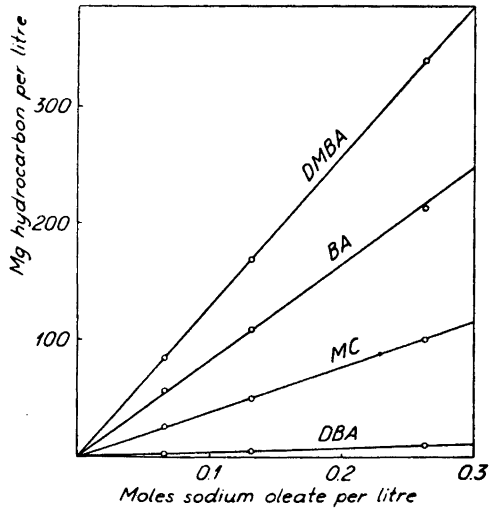

Fig. 2. The solubilities of various polycyclic hydrocarbons in aqueous sodium oleate solutions.

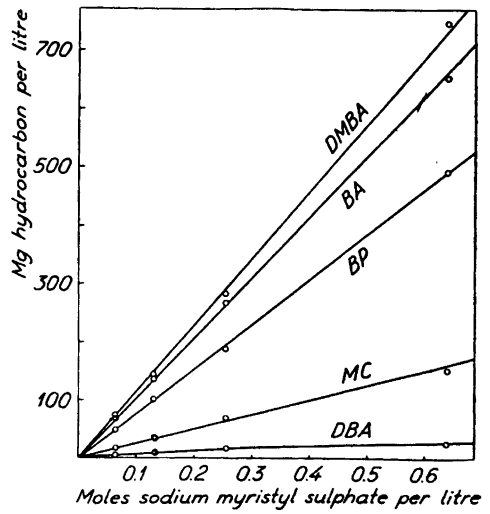

Fig. 3. The solubilities of various polycyclic hydrocarbons in aqueous sodium myristyl sulphate solutions.

linearly with the concentration of the colloid in the case of the paraffin-chain salts investigated (potassium myristate, sodium oleate, sodium lauryl sulphate and sodium myristyl sulphate). The same is the case also in solutions of the non-ionic alkyl aryl polyether alcohol "Triton N", of the polyoxyethylene sorbitan monopalmitate "Tween 40", and of the cationic colloid mixture "Quatrogan". In the solutions of the bile acid salts, on the other hand, the solubilities increase slowly in the beginning, but gradually the solubility increases more rapidly until at the higher colloid concentrations they tend to approach the linear dependence observed in other solutions. Within the range in which a linear relation between the solubility and the colloid concentration exists, all the solutions may be diluted without any precipitation of the carcinogen.

Different colloids differ considerably in their ability to solubilize the same hydrocarbon. In the case of the anionic paraffin-chain salts this ability increases on the whole with the length of the carbon chain. However, the nature of the ionic end group also seems to have an influence, as shown by the fact that sodium lauryl sulphate has a greater solubilizing power than potassium myristate. The alkyl aryl polyether alcohol "Triton N" is considerably superior in its solubilizing power to these anionic association colloids. For the

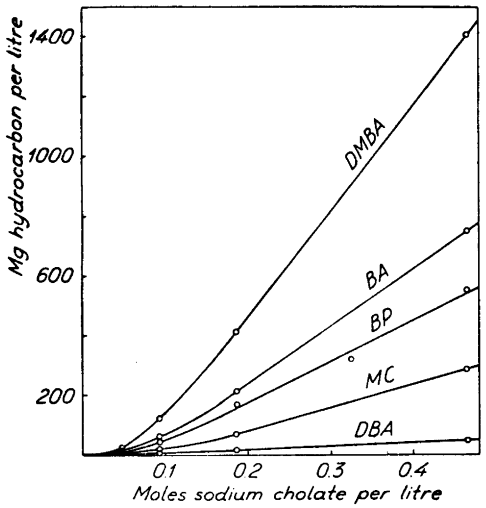

Fig. 4. The solubilities of various polycyclic hydrocarbons in aqueous sodium cholate solutions.

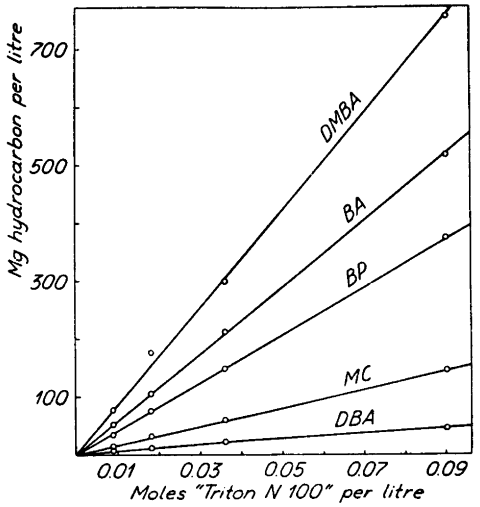

Fig. 5. The solubilities of various polycyclic hydrocarbons in aqueous "Triton N" solutions.

present we do not know the size of the alkyl group in this colloid. Its average molecular weight 710, however, points to a long hydrocarbon chain. At higher concentrations also the bile acid salts surpass the paraffin-chain salts in their solubilizing power and deoxycholate has even a greater solubilizing power than "Triton N", when solutions containing the same weight per cent of colloid are compared. Deoxycholate solubilizes considerably more than cholate. This is partly due to the fact that micelle formation in deoxycholate begins at a much lower concentration than in cholate solutions (the respective critical concentrations are 0.006 *M* and 0.018 *M* [2]). But as will be seen later, the deoxycholate micelles themselves are able to solubilize more carcinogen per mole than the cholate micelles. The power to solubilize carcinogenic hydrocarbons thus increases with decreasing number of OH-groups in the bile acid molecule. It seems therefore probable that the salts of lithocholic acid and the cholanic acids have a still greater solubilizing power. The conjugation of taurine to cholic acid to give taurocholic acid does not seem, as far as our study shows, to have an appreciable influence on the solubilizing power.

A comparison of the ability of the association colloids to solubilize polycyclic aromatic hydrocarbons and their ability to solubilize low molecular

Table 1. The solubilities of polycyclic hydrocarbons in aqueous solutions of different association colloids in mg hydrocarbon per litre association colloid solution. Colloid concentrations in weight per cent.

Aqueous colloid solution	DBA	MC	BP	BA	DMBA
Potassium myristate 20 %	23.7	137			428
» » 8 »	10.1	54.0	118		169
» » 4 »	5.2	25.0	59.9	83.8	86.9
» » 2 »	2.0	11.2			41.7
Sodium oleate 8 %	10.0	101	—	213	338
» » 4 »	5.1	50.1	—	109	169
» » 2 »	2.7	26.3	—	56.5	84.9
Sodium lauryl sulphate 20 %				721	644
» » » 8 »				287	250
» » » 4 »		42.4	48.0	144	119
» » » 2 »		18.2		72.1	63.3
Sodium myristyl sulphate 20 %	24.5	149	492	653	749
» » » 8 »	15.1	70.0	188	268	283
» » » 4 »	8.0	33.6	101	138	144
» » » 2 »	3.5	17.7	48.8	68.2	73.1
Sodium cholate 20 %	48.3	286	553	764	1 409
» » 8 »	14.3	67.9	167	212	413
» » 4 »	3.8	17.6	43.0	63.0	121
» » 2 »	0.9	4.2	10.0	13.5	23.7
Sodium deoxycholate 20 %		677			2 082
» » 8 »		269			1 106
» » 4 »		113			410
» » 2 »		33.0	63.1	73.5	119
Sodium taurocholate 4 %		13.1	33.7		94.4
» » 2 »		3.4			18.7
» » 1 »		0.7		2.6	2.7
"Quatrogan" 10 %					1 150
» 5 »					620
» 2 »					255
"Triton N 100" 20 %			1 143		
» » » 6.4 % *	44.8	145	373	516	755
» » » 2.56 »	22.2	59.8	148	201	299
» » » 1.28 »	10.3	30.8	74.8	105	160
» » » 0.64 »	5.8	13.4	34.1	51.0	77.4
"Tween 40" 20 %		290			
» » 15 »		208			
» » 10 »		128			
» » 5 »		61.2			
» » 2.5 %		27.3			

* 6.4 per cent "Triton N 100" corresponds to 20 per cent "Triton NE".

hydrocarbons, *e. g.* benzene, xylene, hexane, *etc.*[3] *, reveals the following similarities and differences: Independant of the nature of the hydrocarbon the solubilization begins at a definite concentration of the colloid-forming substance, *viz.* at its critical concentration for micelle formation. Above this concentration, the solubilization of benzene, xylene, *etc.*, passes first through a transition range where the solubility of hydrocarbon per mole association colloid increases [3]. This range is relatively narrow for the paraffin-chain salts, but wider in the case of the bile acid salts [2]. Then there follows a range in which the solubility of the hydrocarbon increases linearly with the colloid concentration, the increase in solubility per added mole colloid remaining constant [3, 2]. We have found the mentioned transition range of the solubilization of polycyclic hydrocarbons to occur only in solutions of the bile acid salts. In the other colloid solutions, the solubilities of these hydrocarbons increase linearly from the critical concentration. It may further be pointed out that in the solubilization of the low molecular hydrocarbons by concentrated solutions, the solubility of the hydrocarbon per mole colloid begins to increase when a certain limiting concentration, "the second critical concentration", is exceeded, at least in solutions of paraffin-chain salts [3, 4]. Contrary to this, no increase in the solubility of the polycyclic hydrocarbons per mole colloid was observed at higher concentrations, although the measurements were extended over a fairly broad concentration range. The linear course of the solubility curves predominates, especially with the paraffin-chain salts.

From the slope of the linear part of the solubility curve, it is possible to calculate the maximum solubilizing power ("the saturation capacity") of the micellar substance for the hydrocarbon in question [3]. These values are given in Table 2. From these values we have calculated the number of molecules micellar substance required to solubilize one molecule hydrocarbon.

Table 2 shows that the micelles of "Triton N" have the greatest solubilizing power, followed by the deoxycholate and cholate micelles.

The number of molecules of the micellar substance required for the solubilization of one molecule of a polycyclic hydrocarbon varies considerably for different colloids and for different hydrocarbons. As a rule more than 200 molecules of the paraffin-chain salts are required. This value, however, increases to 8 000—9 000 molecules of the micellar substance per molecule of

* Previously one of us (Ekwall) has quantitatively investigated the solubilization of benzene, toluene, *p*-xylene, *p*-cymene, naphtalene, phenantrene, diphenyl, cyclohexane, and hexane in sodium oleate solutions at 20° C and *p*-xylene in sodium caprinate and sodium laurate solutions at 20° C and in sodium laurate, myristate, oleate, myristyl sulphate, cholate, and deoxycholate solutions at 40° C. The solubilities have been determined with a Zeiss-Löwe interferometer. Some of the results are mentioned in references (3) and (2).

Table 2. The maximum solubilizing power of micellar substances for polycyclic hydrocarbons.

Association colloid	Mg hydrocarbon per mole micellar substance					Moles micellar substance per mole solubilized hydrocarbon				
	DMBA	BA	BP	MC	DBA	DMBA	BA	BP	MC	DBA
Potassium myristate	578	564	398	182	33	445	405	629	1 460	8 470
Sodium oleate	1 320	825	—	379	32	191	278	—	710	8 700
Sodium lauryl sulphate	930	1 046	350	300		308	218	725	890	
Sodium myristyl sulphate	1 142	1 095	780	278	60	225	208	324	960	4 630
"Triton N"	8 340	5 830	4 200	1 650	630	31	39	61	163	440
Sodium cholate	4 057	1 860	1 800	735		63	123	140	385	
Sodium deoxycholate	7 200					36				

the hydrocarbon in the case of the poorly soluble 1,2,5,6-dibenzanthracene. Various methods have shown that in the range just above the critical concentration the micelles of the colloids in question are composed of about 50—200 molecules, and that the number of molecules somewhat increases when hydrocarbons are solubilized by the micelles [3–5]. Our results thus indicate that there can hardly be more than one molecule of polycyclic hydrocarbon per micelle in these solutions *. — In the case of bile acid salts and "Triton N", a much smaller number of molecules in the micellar state is sufficient to solubilize one molecule of the hydrocarbon. Whether there is more than one molecule (from one to four molecules) of the hydrocarbon present per micelle can not yet be decided because the sizes of the micelles of these substances are not known.

* In this connection we do not wish to discuss how to account for the large number of moles micellar substance per mole solubilized carcinogen, *e. g.* in solutions of DBA. It may, however, be suggested that it is scarcely possible that in presence of DBA micelles containing from 4 000 to 8 000 molecules are formed at the critical concentration in solutions of paraffin-chain salts. We are rather inclined to the interpretation that an equilibrium exists between micelles of moderate size containing the polycyclic hydrocarbon and normal micelles which are as such unable to solubilize the large hydrocarbon molecules. This hypothesis is at the present the subject of further examination.

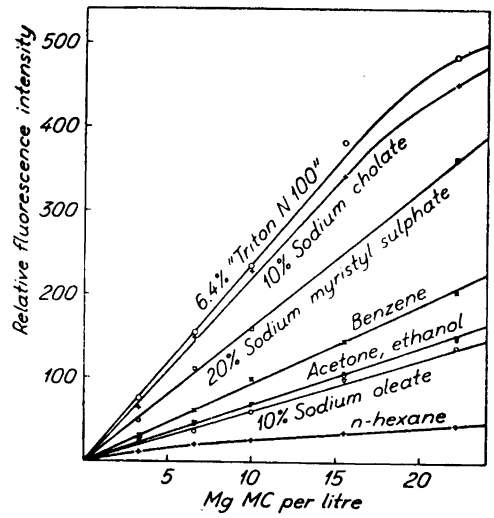

Fig. 6. The relative fluorescence intensity of 20-methylcholantrene in various solvents.

The preceding data indicate that the carcinogenic hydrocarbons are present in a highly-dispersed state in the solutions in question, each molecule being separated from the others and embedded within the hydrocarbon parts of the micelles. These solutions can thus not be compared with the previously used suspensions of the carcinogens which contain small suspended hydrocarbon crystals and which are unstable systems.

The solubilities of the different hydrocarbons in the same colloid differ greatly. For the four hydrocarbons which possess carcinogenic properties, the solubilities increases in the following order: 1,2,5,6-dibenzanthracene < 20-methylcholantrene < 3,4-benzpyrene < 9,10-dimethyl-1,2-benzanthracene. The carcinogenic activity increases on the whole in the same order. In Berenblum's scale[6] concerning painting on the skin, these four hydrocarbons have been given the following ciphers: VI, VIII, VIII, X. 1,2-benzanthracene, which has no carcinogenic properties, is approximately as soluble as the most active hydrocarbon, 9,10-dimethyl-1,2-benzanthracene. Although an immediate connection between the solubilities of the hydrocarbons in the micelles and their carcinogenic activity scarcely can be expected, it seems that a greater solubility assists the penetration of the carcinogen into the tissues and cells of the living organism and thus increases the possibility of the carcinogen for exerting its effect.

The hydrocarbons exhibit a stronger fluorescence in most of the colloid solutions than in the usual solvents. This is seen from Fig. 6, which shows the relative intensity of the fluorescence of 20-methylcholantrene in different

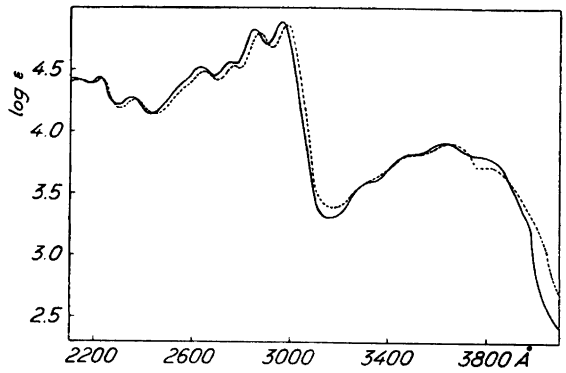

Fig. 7. The absorption spectra of 9,10-dimethyl-1,2-benzanthracene in absolute ethanol (————) and in a 10 per cent aqueous sodium lauryl sulphate solution (– – – – –).

solvents. A benzene solution containing 10 mg 20-methylcholantrene per litre has been used as a reference solution (relative fluorescence = 100). In solutions of "Triton N" and sodium cholate the fluorescence of 20-methylcholantrene is about four times stronger than in acetone and ethanol. Of the colloid solutions studied only in the oleate solutions is a weaker fluorescence (approximately as strong as in ethanol) observed. It may also be pointed out that oleate solutions of 3,4-benzpyrene become brownish and the fluorescence has a greenish shade, which points to a chemical change undergone by the hydrocarbon. An investigation of whether this observation and the weaker fluorescence of the other hydrocarbons in oleate solutions is possibly due to oxidation induced by the oleate is in progress.

An investigation of the absorption of the polycyclic hydrocarbons dissolved in solutions of association colloids indicated that their absorption spectra remain almost unchanged. In all cases, however, a slight shift towards the longer wavelengths has been observed (Fig. 7)*.

BIOLOGICAL EXPERIMENTS

We have conducted biological experiments to determine whether these aqueous association colloid solutions of carcinogenic hydrocarbons can be employed in the study of chemical carcinogenesis. The main results of this work are outlined below.**

* The absorption spectra of these hydrocarbons in different association colloid solutions, as well as the strong fluorescence of these solutions, is the subject of a detailed investigation which has not yet been completed.

** These experiments are described in detail elsewhere [7-12, 19, 20, 22, 23].

The aqueous solutions of association colloids have a low surface tension and make a very small contact angle with lipid surfaces. They therefore easily wet and penetrate such surfaces. By painting the skin of mice with solutions of carcinogenic hydrocarbons in these solvents, we have found that the solutions rapidly penetrate into the skin and transport the solubilized hydrocarbon into the tissues. A short time after the application of the solution the fluorescent carcinogen may be observed with a fluorescence microscope in the keratinized layers of the epidermis, in the sebaceous elements, and in the fat deposits of the skin [1, 9, 12].

Furthermore, we have found that these solutions, when introduced into the stomachs of mice, penetrate into the cells of the walls of the forestomach [11] and into a kind of fine network in the outer muscle layer of the stomach [14]. In this respect the aqueous carcinogen-association colloid solutions are similar to the entirely lipid solvents (*e. g.* paraffin oil), and lipophilic-hydrophilic solvents such as polyethylene glycols ("Carbowaxes") *etc.*[13, 14].

Until quite recently it has not been definitely observed that the carcinogenic hydrocarbon penetrates into the cells of the glandular stomach. We have, however, shown that such a penetration takes place when the carcinogen is dissolved in anhydrous polyethylene glycols ("Carbowaxes") [13], and in other solvents possessing both marked lipophilic and hydrophilic properties, such as anhydrous non-ionic association colloids (alkyl aryl polyether alcohols *etc.* [14, 19, 22]). Also when the carcinogen is dissolved in aqueous solutions of association colloids, *e. g.* a 70 per cent sodium taurocholate solution *, 90—20 per cent "Triton N" solutions *etc.*, a penetration into the wall of the glandular stomach can be observed [14]. In some cases a very faint and shortly disappearing fluorescence in the superficial layer has been observed also when more dilute colloid solutions were used. Whether this is to be interpreted as a sign of penetration of the carcinogen has not as yet been cleared up.

Experiments with mice have revealed that the polycyclic hydrocarbons retain their carcinogenic activity when solubilized in the aqueous solutions of association colloids of different types [7–12, 19, 20, 22, 23]. Representatives of widely different association colloids such as the non-ionic association colloids with neutral micelles (alkyl aryl polyether alcohol), anionic association colloids with negatively charged micelles (fatty acid salts, alkyl sulphates, bile acid salts), and cationic association colloids with positively charged micelles (cetyl trimethyl ammonium salts, alkyl dimethyl benzyl ammonium salts, cetyl

* When BP is solubilized in a synthetic bile containing taurocholate, oleate, cholesterol, and lecithin, it penetrates into the superficial layers of the wall of the glandular stomach. The same result is obtained with an emulsion of arachidic oil and natural bile containing BP (14).

pyridinium salts) have been used. Malignant cutaneous tumours have been produced in mice by carcinogens solubilized in all these colloid solutions when painted on the skin. Malignant tumours have also been produced in the forestomach of mice by feeding carcinogens solubilized in solutions of the first two types of association colloids. Cutaneous tumours have been caused by a concentration of 9,10-dimethyl-1,2-benzanthracene as low as 0.0045 per cent in aqueous lauryl sulphate solutions. Tumours in the forestomach have developed after the administration of only 0.6—1.0 mg carcinogen in different colloid solutions. This and other observations [11] may be considered to indicate that these solutions facilitate the action of the carcinogen.

The type and concentration of the association colloid seem to have a certain effect on the carcinogenesis [9,11,12]. A systematic investigation of the significance of this "solvent effect" is in progress.

DISCUSSION

In the experimental study of the chemical carcinogenesis induced by polycyclic hydrocarbons one has to introduce the fat-soluble, but practically water-insoluble, carcinogens into the tissues and cells with their aqueous systems. The introduction of carcinogens in aqueous association colloid solutions may therefore in certain cases be more advantageous than the use of their solutions in purely lipophilic fats and oils, or in organic solvents such as benzene and acetone which are foreign to the living organism, and the use of their aqueous suspensions. Owing to their aqueous character and since they, because of the presence of micelles, also possess lipophilic properties, these solutions approach in some respects the conditions prevailing in the living cells. As known, there are in the living organism many substances possessing properties more or less typical of the association colloids: fatty acid and bile acid salts, some bile acids themselves, phosphatides and cerebrosides etc. The solubilization mechanism studied thus provides a model solubilization and transport mechanism for water-insoluble carcinogens which may also apply in principle within the living organism.

From a methodical point of view, the use of aqueous association colloid solutions of carcinogens offers many advantages in the experimental study of the chemical carcinogenesis [9, 18, 19, 21, 22] *. As we have seen, these solutions are homogeneous and stable and the carcinogen is dispersed in them to the highest possible degree. In many cases they permit a high dilution without any precipi-

* In many respects these aqueous solutions offer advantages similar to solutions of the hydrocarbons in the anhydrous water-soluble polyethylene glycols ("Carbowaxes") [13–17, 19, 22].

tation of the carcinogen, which offers possibilities for the carcinogen to remain in solution also in the tissues provided that the micelles are stable there. With the aid of these colloid-forming substances it is possible to vary the chemical and physico-chemical character of the solution within wide limits. The carcinogen can be administered solubilized in neutral, in positively, or negatively charged micelles. On the other hand, the carcinogen can be solubilized in micelles which are stable at the hydrogen ion concentration of the biological environment or in micelles which are broken up at a given pH so that the carcinogen is precipitated; these possibilities can be of interest especially in the study of the behaviour of the carcinogens in the alimentary canal. Because these aqueous solutions are not appreciably volatile, the carcinogen concentrations of the solutions remain constant. This fact and the simplicity of dosing these solutions facilitate quantitative experiments. The exceptionally strong fluorescence of the carcinogens in these solutions may also be advantageous in the study of the first stages of their penetration. Because the solutions possess both lipophilic and hydrophilic properties it is possible to introduce at the same time other fat-soluble or water-soluble substances and to study their co- or anti-carcinogenic action [17].

The biological experiments have shown that these solutions are appropriate for application by painting on the skin and that in feeding experiments they offer considerably greater possibilities than the solutions employed earlier for varying and defining the experimental conditions. For subcutaneous [24] and intravenous injection the possibilities are more limited: at the present it seems that most colloids can be used only in relatively dilute solutions. A few of them can, however, be injected intravenously in fairly high concentrations [25].

As mentioned above, the surface chemical and colloid chemical properties of the association colloids assist the penetration of carcinogens into the tissues and cells, and they perhaps also promote their spreading in the organism. We are paying particular attention to the role of the solvent in the stages which precede the carcinogenesis, but we are also studying the significance of the solvent for the actual carcinogenic process.

An investigation with such a purpose provides a greater interest through the already mentioned fact that the living organism itself contains substances that are in principle of the same type as the solvents we have used. The bile, for example, is an association colloid solution which is known to solubilize carcinogens. Although we have not as yet been able to show that carcinogens in natural bile penetrate into the wall of the glandular stomach, one can not dismiss the possibility that bile which accidentally enters the stomach can, at least under certain conditions, assist the penetration of a carcinogen which has entered the stomach, *e. g.* along with food. The study of the

role of the association colloids in the first stages of the chemical carcinogenesis is of interest also because substances of this type are coming into use nowadays in ever increasing amounts.

SUMMARY

The solubilities of five polycyclic hydrocarbons in aqueous solutions of several association colloids of different types have been determined. In all these colloid solutions, except those of bile acid salts, the solubilities increase linearly with the colloid concentration from the critical concentration up to high concentrations. The maximum solubilizing power ("the saturation capacity") of the micellar substance for the different hydrocarbons has been calculated. The values obtained indicate that the hydrocarbons in these solutions are dispersed to a very high degree, apparently with only one molecule per micelle. The intensity of the fluorescence of the hydrocarbons is considerably greater in these solutions than in the usual solvents. Except for a slight shift towards longer wavelengths, their absorption spectra remain almost unchanged.

Animal experiments have revealed that a carcinogenic hydrocarbon solubilized in these solutions easily penetrates through the skin of mice. It also penetrates into the wall of the forestomach of mice and at least when the colloid concentration is not too low, also into the wall of the glandular stomach. The hydrocarbons maintain their carcinogenic activity in these solutions independent of the chemical structure and the physico-chemical properties of the colloid. Malignant tumours have been produced in the skin and in the forestomach of mice. Tumours have been caused by minute amounts and very dilute solutions of the carcinogen.

The advantages of the aqueous association colloid solutions in the study of chemical carcinogenesis are discussed.

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