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On the Solubilization of Carcinogenic Hydrocarbons by Association Colloids

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The polycyclic aromatic hydrocarbons that possess carcinogenic properties are fat-soluble, but are practically insoluble in water. When homogeneous solutions of these substances have been needed in experimental cancer research it has been found necessary to dissolve them in liquids such as benzene, acetone, sesame oil, tricaprylene etc.[1]. In some cases suspensions of the carcinogenic hydrocarbons in water or in glycerol have been employed. Attempts have been made to disperse these substances in as fine a state and as stably as possible; for instance by letting an acetone solution of the hydrocarbon to drip into distilled water and then removing the acetone by dialysis into pure water [2] (Feigenbaum), or by rapid evaporation at low pressures [3] (Weigert and Mottram). Several investigators have stabilized the suspension with the aid of a colloid. Berenblum [4] and Boyland [5] started with a pyridine solution of the hydrocarbon and diluted it carefully with an aqueous solution of acacia gum, after which they removed the pyridine by dialysis, whereas O'Hara and Pollia [6] dialyzed the pyridine solution directly into the aqueous acacia gum solution. Lorentz and Andervont [7], Shimkin [7] and others used horse serum, Lasnitzki and Woodhouse [8] gelatin as the stabilizing colloid. Stamer [9] produced suspensions by means of dissolving the carcinogenic hydrocarbon in melted »Postonal», a derivative of poly-ethylene oxide and then stirring the mixture in hot distilled water. Thus suspensions were obtained containing up to 2.5 % 1,2,5,6-dibenzanthracene. The suspensions were, however, unstable and had to be used within one hour. — Winterstein and Vetter [10] treated 1,2-benzpyrene in acetone with a 20 % aqueous solution of sodium desoxycholate and found that after removal of the acetone, the hydrocarbon

had dissolved to the extent of 2 mg per ml. Fieser and Newman [11] investigated the formation of molecular compounds of desoxycholic acid with some polynuclear aromatic hydrocarbons and found that in absolute alcohol solutions of the components crystals of »choleic acids» containing four molecules of desoxycholic acid and one molecule of methylcholantrene or 1,2,5,6-dibenzanthracene were formed. The sodium salts of these choleic acids are soluble in water *. — Beck [12] added an ether solution of a carcinogenic hydrocarbon to a highly concentrated solution (1.8 g/ml) of an ether soap, the identity of which was not disclosed. This comprised the storage solution, and solutions of desired concentrations were prepared by diluting with water and vaporizing the ether. *E. g.* solutions containing 7.5 % by weight of soap and 0.1 mg per ml of hydrocarbon were prepared in this manner. The fluorescence spectra of the hydrocarbon in these aqueous solutions and in acetone solution were identical. However, these solutions were unstable, for even within a few hours the limpid solutions were dimmed by opalescence. But Beck found that even in this state the hydrocarbon particles were much smaller than those present in the colloid solutions previously used. Graffi [13] mentions incidentally that the sodium salts of the higher fatty acids are able to dissolve benzpyrene to a certain extent. — Recently Weil-Malherbe [14] found that the solubility of carcinogenic hydrocarbons is considerably increased in the presence of purines, and that the solubilizing effect of the purines varies with their molecular structure. Apparently the solutions are homogeneous, but the solubilization process seems to result in a quenching of the fluorescence characteristic of the carcinogenic hydrocarbon.

As it would be advantageous in certain cases if carcinogenic hydrocarbons could be handled in the form of their stable and absolutely homogeneous aqueous solutions we have investigated the possibility of solubilizing these hydrocarbons with the aid of association colloids. A characteristic of these colloids, among which common soap is the best known type substance, is that when they are dissolved in water their molecules and ions aggregate when a certain concentration is exceeded to form micelles that are able to solubilize considerable amounts of fat-soluble substances such as hexane, cyclohexane, benzene, toluene *etc.* Also naphthalene, anthracene and phenanthrene are solubilized in slight amounts by these colloids [15]. We have now found that carcinogenic hydrocarbons, in spite of the rather large size of their molecules, dissolve in detectible amounts in these colloids yielding clear, absolutely homogeneous and stable, more or less fluorescent, aqueous solutions [16].

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* Lately Weil-Malherbe reported experiments conducted with sodium deoxycholate-water solutions of benzpyrene (*Brit. Journ. of Cancer* **1** (1947) 423).

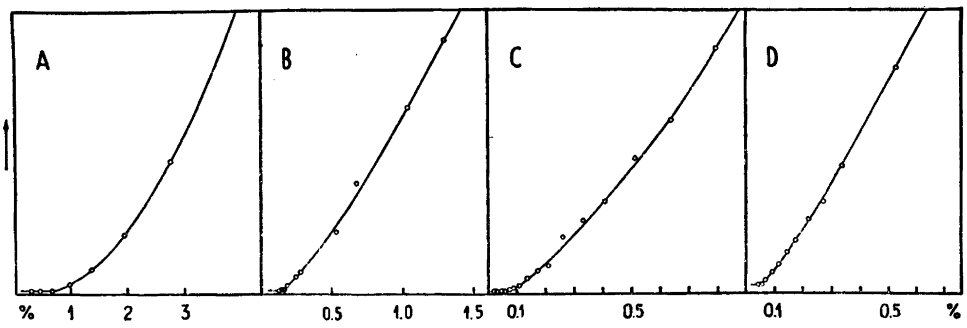

Fig. 1. The fluorescence of colloid solutions saturated with 20-methylcholanthrene. The relative values of the fluorescence intensity as measured with a Pulfrich Step-photometer employing one of the solutions as reference solution has been plotted against the concentration of the colloid in the solution in weight per cent. A. Sodium cholate; B. Sodium myristate; C. Sodium myristyl sulphate; D. Triton NE.

Several colloid-forming substances have been investigated respecting their ability to solubilize carcinogenic hydrocarbons: *e. g.* sodium oleate and myristate, sodium myristyl sulphate, sodium cholate and the commercial compounds Mersolate, Gardinol WA and Triton NE. Mersolate is an alkyl sulphonate, Gardinol is probably an aliphatic alcohol sulphonate and Triton NE is an alkyl polyether alcohol, the last-mentioned differing from the others in being a non-ionogenic association colloid. The colloid solutions were shaken with the finely powdered hydrocarbons in a thermostat at 40° C until the solutions were saturated with the hydrocarbons. The solubilities of the following three hydrocarbons have been investigated: 20-methylcholanthrene, 9,10-dimethyl-1,2-benzanthracene and 1,2,5,6-dibenzanthracene.

The solubilization ability of the association colloid becomes evident only when its concentration exceeds a definite limit, see Fig. 1. The figure illustrates the fluorescence of solutions of sodium cholate, myristate, myristyl sulphate and Triton which have been saturated with methylchloranthrene. In dilute solution the fluorescence is nil, as in the pure colloid, but at a definite concentration fluorescence appears and the intensity of the fluorescence increases rapidly, indicating a corresponding increase in the solubility of the hydrocarbon. The inception of the fluorescence coincides with the attainment of the critical concentration for micelle formation of the association colloid; at this point the colloidal substance begins to develop. This implies that the solubilization of the polycyclic hydrocarbon is coupled with the formation of micelles, and leads one to suppose that the rather large hydrocarbon molecules are enclosed in the hydrocarbon part of the micelles as are benzene *etc.*

Table 1. Approximate solubility values of the carcinogenic hydrocarbons in mg per 1000 ml solution. Colloid concentration in weight per cent.

	2 %	4 %	8 %	20 %
	Sodium cholate solutions			
1,2,5,6-Dibenzanthracene	0.84	3.9	14	
20-Methylcholanthrene	4.3	17	65	390
9,10-Dimethyl-1,2-benzanthracene	27	145	450	
	Sodium oleate solutions			
1,2,5,6-Dibenzanthracene	2.9	5.9	11	
20-Methylcholanthrene	21	45	83	
9,10-Dimethyl-1,2-benzanthracene	81	165	308	
	Triton NE solutions			
1,2,5,6-Dibenzanthracene	5.5	11	22	
20-Methylcholanthrene	15	33	59	157
9,10-Dimethyl-1,2-benzanthracene	89	180	360	700—1100

The method nearest at hand for the determination of the solubilities of the hydrocarbons is the measurement of their fluorescence intensity. A linear relation between the intensity of the fluorescence and the hydrocarbon concentration is however to be found only at comparatively low concentrations (*e. g.* up to ca. 40 mg methylchloranthrene per liter in 8 % sodium cholate solutions). Also the intensity is affected by many factors such as the solvent's own absorption, the quenching effect of the fluorescent substance, of the colloid and of other foreign substances (*e. g.* oxygen). Consequently identical hydrocarbon concentrations may give rise to fluorescence of varying intensity in different solvents, *i. e.* in solvents containing various concentrations of one colloid or in solvents containing different colloids. It seems that in some cases the hydrocarbon itself is changed in some way during the solubilization or during the storage of the solutions. We have not as yet been able to overcome these difficulties, which affect the determination of the concentration. Furthermore, the instrument at our disposal (Pulfrich Step-photometer) has not enabled us to attain the desired accuracy in the fluorescence measurements. Hence the solubility values given above are to be regarded only as approximate.

The values given in Table 1 confirm that the solubilities of the hydrocarbons increase with the concentration of the colloid. There is, however, a marked difference between the increase in the solubility in the sodium oleate and Triton solutions on one hand, and in the sodium cholate solutions on the other. In the former group the solubility increases in the same proportion as

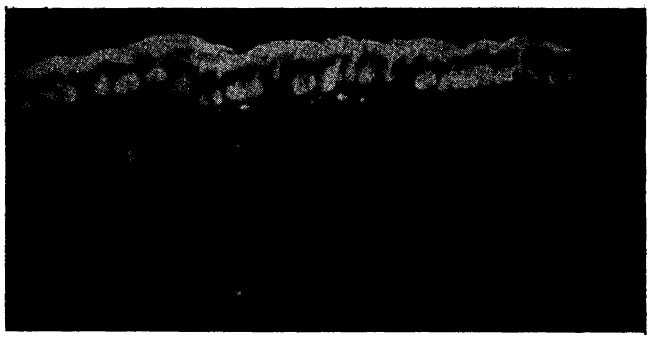

Fig. 2. A microphotograph taken in ultraviolet light of a frozen section from the skin of a mouse painted with a 20 % Triton NE solution saturated with 20-methylchloranthrene, unstained section. The specimen was taken 1 hour and 45 min after the painting of the skin. The fluorescent substance is clearly visible.

the concentration of the colloid, a behaviour that is also found in the case of the solubility of lowmolecular hydrocarbons in these and other association colloids over a wide concentration interval (in the range of »small micelles», »Kleinmicellen»). In the cholate solutions the increase in solubility is more rapid; there is a practically linear relation between the solubility and the quadrate of the cholate concentration. A consequence of this is that the solubility of the hydrocarbon in the more concentrated cholate solutions is higher than in the corresponding oleate and Triton solutions, whereas the reverse is true in the more dilute solutions. Whether this behaviour is due to the different manner in which the colloid is formed from the substances with straight chains as compared with its formation from the cholate with its four-ringed molecule and three hydroxyl groups, or to differences in the solubilization mechanism, is a question that must be left open.

The solubilities of the three hydrocarbons in the same colloid differs widely. In all cases 1,2,5,6-dibenzanthracene shows the slightest and 9,10-dimethyl-1,2-benzanthracene the highest solubility. The greatest variations are found in the cholate solutions. Also in benzene the first-mentioned hydrocarbon is the least and the last-mentioned the most soluble. It is interesting to note that 1,2,5,6-dibenzanthracene has the least carcinogenic effect, while 9,10-dimethyl-1,2-benzanthracene has proved to be in many cases the most active in this respect. In the fifteen-step scale introduced by Berenblum [17] 9,10-dimethyl-1,2-benzanthracene is characterized by the cipher X, 20-methylcholanthrene by VII and 1,2,5,6-dibenzanthracene by VI when skin tumours are concerned. It remains, however, to be found whether a relation exists

between the different solubilities of the hydrocarbons in the micelles and their carcinogenic action.

Experiments with animals have been undertaken to study the effect of the association colloid solutions of the carcinogenic hydrocarbons. When a small amount of such a solution is painted on the skin of a mouse the carcinogene is rapidly absorbed and with the aid of a fluorescence microscope the hydrocarbon has been found distributed in about the same places as when applied by painting with an acetone solution of the carcinogene. Fig. 2 is a microphotograph of a cut from the skin after painting with methylchloranthrene in Triton solution. The fluorescent carcinogene is found in the epidermis, and in the sebaceous glands with their contents. We have also been able to produce skin tumours by application of these association colloid solutions of the hydrocarbons (unpublished data).

The experiments are being continued in several directions. Animal experiments in which association colloid solutions of the carcinogenes are being introduced by subcutaneous and intravenous injection and with food are in progress.

SUMMARY

When the concentrations of association colloids of a number of types exceed their respective so-called critical concentrations for micelle formation they are able to bring carcinogenic hydrocarbons into solution. The solutions obtained are clear, absolutely homogeneous and stable. In some cases the solubilities of the hydrocarbons increase in the same proportion as the concentration of the colloid (oleate, Triton NE) and in other cases as the quadrate of the colloid concentration (cholate). There is some parallelism between the solubilities of the three hydrocarbons investigated in a given colloid and their carcinogenic action.

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