XXXVIII. CHEMICAL CONSTITUTION IN RELATION TO THE PRECIPITATION REACTIONS OF NORMAL SERUM WITH LIPOID SUSPENSIONS

By CAMERON GORDON ANDERSON

From the Bacteriology Department, University of Edinburgh

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THE precipitation reactions of normal serum with a variety of lipoids extracted from bacteria, animal tissue etc. were shown by Mackie & Anderson [1937] to fall into five types, designated A_1 , A_2 , B , C and D . The A_1 type of reaction is characteristic of syphilitic sera and of the normal sera of certain animals interacting with crude alcoholic or chloroform extracts of heart muscle and with separated phosphatides. The serum factor concerned in this reaction is thermolabile, being annulled by heating at 65° for 30 min. The precipitation is restricted (in the method used for these tests) to a range of serum dilutions from 1 in 4 to 1 in 64. Reaction A_2 corresponds to A_1 in the thermolability of the serum factor and in dilution-range but occurs also with normal human sera, and is given by fats and acetone extracts of heart muscle and of various bacteria. The serum factor of the B reaction has the same thermolability as that of the A reactions, but this effect takes place in a higher zone of serum dilutions. It is given by the fats and the acetone extracts. The C reaction differs from A and B in the thermostability of the serum factor which resists heating at 90° for 30 min. Like the B effect it occurs in a high range of serum dilutions. In unheated normal serum it is apparently inhibited and is only manifested in sera which have been heated above 75° . The reaction occurs mainly with fats and acetone extracts but is also sometimes given by phosphatides. The D reaction is analogous to the C reaction but occurs in still higher dilutions of serum.

From preliminary observations mentioned in the above paper it seemed possible that the lipid factors concerned in these precipitation reactions might be associated with definite compounds, and also that a given compound might react in more than one way, giving rise to one or more of the five types of reaction. This question has now been investigated with a large range of well-defined compounds in pure form, all characterized by solubility in alcohol and insolubility in water.

The compounds tested were selected to contain different characteristic groups, both alone and in various combinations with one another in a single compound. Homologous series of increasing mol. wt. for each type of compound were included in order to determine, in some measure, the influence of decreasing proportions of the "active" group of the molecule. The following classes of compound were examined: paraffins, olefins, primary and secondary alcohols, saturated mono- and dibasic acids, unsaturated acids, salts, esters, glycerides and sterols.

Methods

The compounds were purified by appropriate means (recrystallization, fractional distillation etc.) and dissolved in alcohol, containing 0.15% cholesterol, to give 1% solutions. In cases where the solubility was less than 1% at room temperature hot solutions were employed. In many instances it was found that suspensions made as previously described [Mackie & Anderson, 1937] flocculated spontaneously on standing. This difficulty was overcome by using saline containing 0.5% gelatin as the diluent, when, with rare exceptions, the suspensions were stable. It was shown

that neither cholesterol nor gelatin alone gave any visible reaction with serum, under the conditions of the experiments. Sera heated at 55° and at 80° (after fourfold dilution with saline) were used in a series of quadrupling dilutions from 1 in 4 to 1 in 1,048,576; 0.15 ml. of the suspension of the substance under investigation was added to 0.3 ml. of each dilution of serum, the mixture incubated at 37° for 4 hr. and then kept overnight at room temperature before reading the results. A second reading after 48 hr. at room temperature was not recorded, as many of the suspensions showed deposits which confused the reading but were regarded as having no significance. The results were recorded and classified according to the system adopted in the previous investigation. The stability of the suspensions was controlled in every case by including a tube with 0.15 ml. of the suspension plus 0.3 ml. of saline.

Results

These are collected in Table I. The figure following the designation of the various reactions indicates the number of times the corresponding reaction occurred with the particular serum and compound concerned. A symbol such as *BC* 3 signifies that a *B* and a *C* reaction occurred together three times. In the column "animal sera" are collected the results obtained with ox, sheep, rabbit and horse sera which all behaved in closely similar manners.

It will be seen that the paraffins generally gave a negative reaction. *A*₂ and *C* reactions occurred very rarely. The olefins gave no reaction or a very weak *A*₂ reaction, with once a *C* reaction. Carotene was consistently negative. The alcohols, whether primary or secondary, saturated or unsaturated, were negative or gave a weak *A*₂ reaction. Cetyl alcohol was a marked exception in that it very frequently yielded a *B* reaction alone, although sometimes a negative reaction occurred or *A*₂ or *C* reactions either alone or together. The acids almost invariably gave a *C* reaction, usually alone but sometimes in combination with *A*₂, *B* or *D*. The unsaturated acids gave mixed reactions more frequently than the saturated acids, the additional reaction being usually *A*₂. The salts behaved very like the corresponding acids. The esters were generally negative or gave weak *A*₂ reactions occasionally in combination with *C*. In one or two instances *C* occurred alone. Cetyl palmitate (spermaceti) was invariably negative. The glycerides were negative or gave *A*₂ or *C* reactions in the case of the acetins, but more frequently combined reactions with the higher glycerides. Triolein was exceptional in giving no reaction or *A*₂ alone. The sterols were invariably negative. It is interesting to note that none of these simple compounds gives the *A*₁ reaction characteristic of syphilitic sera acting with alcoholic extracts of heart tissue or with phosphatides separated therefrom.

DISCUSSION

From these results it may be inferred that the reactions depend largely on the polar groups present in the molecules. Thus the paraffins which have no polar group yield very weak reactions or none at all. The olefins, having only the weakly polar double bond between carbon atoms, >C=C< , behave in the same way. The hydroxyl group in the alcohols is weakly polar and these substances are again negative or give only weak reactions. The acids and salts, however, which contain the strongly polar carboxyl group give a strong *C* reaction, frequently alone but occasionally with other reactions which are probably to be ascribed to the rest of the molecule, which however is comparatively inactive when compared with the carboxyl group. The esters have much weaker reactions than the acids, the *C* reaction being largely abolished. This appears to be due to the masking of the activity of the polar carboxyl group by the presence of the alcohol residue. With esters of high mol. wt. as in spermaceti, the cholesterol esters, or the esters of the higher fatty acids, this weakening of both *C* and

Table I

	Active group	Sera		
		Human syphilitic	Human normal	Animal
Paraffins:				
<i>n</i> -Heptane	None	Neg. 1	Neg. 1, A1	Neg. 3
Hexadecane		Neg. 7, C1	Neg. 9	Neg. 6
Octadecane		Neg. 4	Neg. 6	Neg. 2
Olefins:				
Hexadecene		Neg. 4, A1	Neg. 5, A1, C1	Neg. 6
Carotene		Neg. 4	Neg. 7	Neg. 3
Alcohols. Primary:				
<i>n</i> -Heptyl alcohol		Neg. 2	Neg. 4	Neg. 2
<i>n</i> -Octyl alcohol		Neg. 1	Neg. 2	Neg. 2
Cetyl alcohol		Neg. 3, B6, AB1, BC3	Neg. 4, B10, AB2, BC4, ABC1	Neg. 3, A3, B3, C1
Alcohols. Secondary:				
<i>sec</i> -Heptyl alcohol		Neg. 1	Neg. 2	Neg. 4
Linalol		Neg. 1	Neg. 1	Neg. 4
<i>sec</i> -Decyl alcohol		Neg. 2, A1	Neg. 4, A3	Neg. 2
<i>sec</i> -Undecyl alcohol		A1	Neg. 1, A2	Neg. 4
Acids. Saturated. Monobasic:				
<i>n</i> -Hexanoic acid		C1	C2	C2
Pelargonic acid		C1	C2	C4
Myristic acid		C3	C2	C1, AC1, CD1
Palmitic acid		C3, CD1	C2, CD3	C3
Stearic acid		C4, CD2	C1, CD4	C1, BC1
Arachidic acid		C2, CD1	C2, CD1	A1, C1 AC1
Acids. Saturated. Dibasic:				
Adipic acid		C3, AC1	C2	C3
Azelaic acid		C2	C1	C2
Acids. Unsaturated:				
Tiglic acid		C2	C4	C3, AC1
Undecylenic acid		C2	C2	C4
Oleic acid		C1, AC2	C2, AC2	AC5
Elaidic acid		AC3, ACD1	C2, AC2, BC1, CD1	C2, AC3, BC2, ACD1
Linoleic acid		C1	C2	A1, BC1
Ricinoleic acid		C3	C6	Neg. 3, A1, C2
Abietic acid		Neg. 1, C3	C4	Neg. 2
Salts:				
Sodium stearate		C4, CD1	C3	A1, C5, AC2, CD3
Sodium oleate		Neg. 4, C2	A1, C2, AC1	Neg. 2, A1, C1, AC4, BC1
Esters:				
Cholesteryl acetate		Neg. 2	Neg. 2	Neg. 4
Cholesteryl oleate		Neg. 2	Neg. 2	Neg. 3, A1
Cholesteryl stearate		Neg. 2	Neg. 2	Neg. 2
Octyl acetate		Neg. 4, C1	Neg. 2, A1, C2, AC1	Neg. 2
Ethyl sebacate		Neg. 1, C1	Neg. 4, C1	Neg. 2
Linolyl acetate		Neg. 2	Neg. 2, C2	Neg. 2
Ethyl palmitate		Neg. 7	Neg. 7, A2	Neg. 10
Butyl oleate		Neg. 3	Neg. 3	Neg. 4
Butyl stearate		Neg. 2	Neg. 3	Neg. 4
Cetyl palmitate		Neg. 2	Neg. 2	Neg. 2
Glycerides:				
Monoacetin		Neg. 3, C1	Neg. 2	Neg. 3, A1
Diacetin		Neg. 2, C2	Neg. 2	Neg. 3, A1
Triacetin		Neg. 3, C1	Neg. 2	Neg. 4
Distearin		C1, AB3, CD1	C2, AB2	AB1, AC1, CD3
Tristearin		Neg. 2, C2	C4, CD1, ACD1	Neg. 1, AC1
Diolen		B2, AB1, AC2	B1, AC2	A1, B2, C1, AC8
Triolein		Neg. 1, A1	Neg. 4, A1	Neg. 2, A1
Tripalmitin		Neg. 1, A1, C3	Neg. 2, A1, C1, CD1	Neg. 1, B1
Sterols:				
Ergosterol		Neg. 2	Neg. 2	Neg. 2
Calciferol		Neg. 4	Neg. 7	Neg. 2
Cholesterol		Neg. 4	Neg. 4	

A_2 reactions is more obvious. Esters containing the lower acids or alcohols are more active, since the degree of masking of the carboxyl group is not so great. This effect of increased mol. wt. in reducing the activity of a compound is also to be observed in the case of the higher paraffins and olefins. Similar behaviour is also exhibited by the glycerides, the triglycerides being less active than the diglycerides. The sterols which are large molecules containing few and weak polar groups (the double bonds and hydroxyl group) are characterized by completely negative reactions. It appears from these results that the C reaction is associated with the $>C=O$ group, especially as it occurs in the carboxyl group and less strongly when the carboxyl group is esterified.

These findings are well illustrated by the various members of the C_{16} series of compounds. The hydrocarbons, hexadecane and hexadecene, are almost completely inactive; cetyl alcohol gives a strong B reaction, palmitic acid a strong C reaction, whilst ethyl palmitate and tripalmitin are fairly weak in their activity and finally cetyl palmitate is quite inactive. The B reaction seems to be associated with an increase in mol. wt., particularly where the polar groups are not very strong, as is the case with cetyl alcohol.

A range of activities which might be regarded as analogous was found by Schulmann & Rideal [1937] who examined the penetrating effects of various substances into monomolecular films. They showed that long chain fatty acids and alcohols (including cetyl alcohol) can penetrate protein films with great ease, forming stable mixed monolayers.

It seems very probable that the strong reactions given by the acids may be due, in part at least, to direct combination between the carboxyl group and the free amino groups of the serum proteins. In the case of other compounds the reactions probably result from the association brought about by residual affinities between the proteins and such groups as $-OH$, $-OR$, $>C=C<$ etc. in the compounds studied. As mentioned by Schulmann & Rideal even the non-polar portion of the molecules may play a part in such association, a fact which accounts for the weak reactions given by the non-polar paraffins. Przyłęcki *et al.* [1935] also observed that non-polar paraffins were strongly adsorbed by serum albumin and other proteins, but weakly adsorbed by casein, gelatin and peptone. They maintain that the adsorption of acids and esters is mainly through the polar groups of the proteins.

SUMMARY

1. Certain precipitation reactions designated A_2 , B , C and D [Mackie & Anderson, 1937], similar to those described with normal serum and various lipid preparations extracted from bacteria, vegetable and animal tissues, are obtained with simple compounds of known constitution all soluble in alcohol and insoluble in water.

2. The degree of activity runs parallel with the strength of the polar groups concerned.

3. Fatty acids give, generally, the C reaction, whilst other substances give either no reaction or the A_2 reaction. Cetyl alcohol, in contrast to the other alcohols, gives a strong B reaction.

4. None of the compounds tested yields the A_1 reaction which is characteristic of the interaction of phosphatides with syphilitic sera and the normal sera of certain animals.

REFERENCES

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