EFFECTS OF FAT-SOLUBLE COMPOUNDS ON LYSOSOMES *IN VITRO*

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INTRODUCTION

The name *lysosomes* has been given to a special group of cytoplasmic particles, first identified in rat liver but now known to occur in numerous animal tissues, and characterized essentially by their content of a variety of acid hydrolases. Our present knowledge concerning these particles has been reviewed in detail by de Duve (1959) and by Novikoff (1961), whose papers should be consulted for references to the original literature.

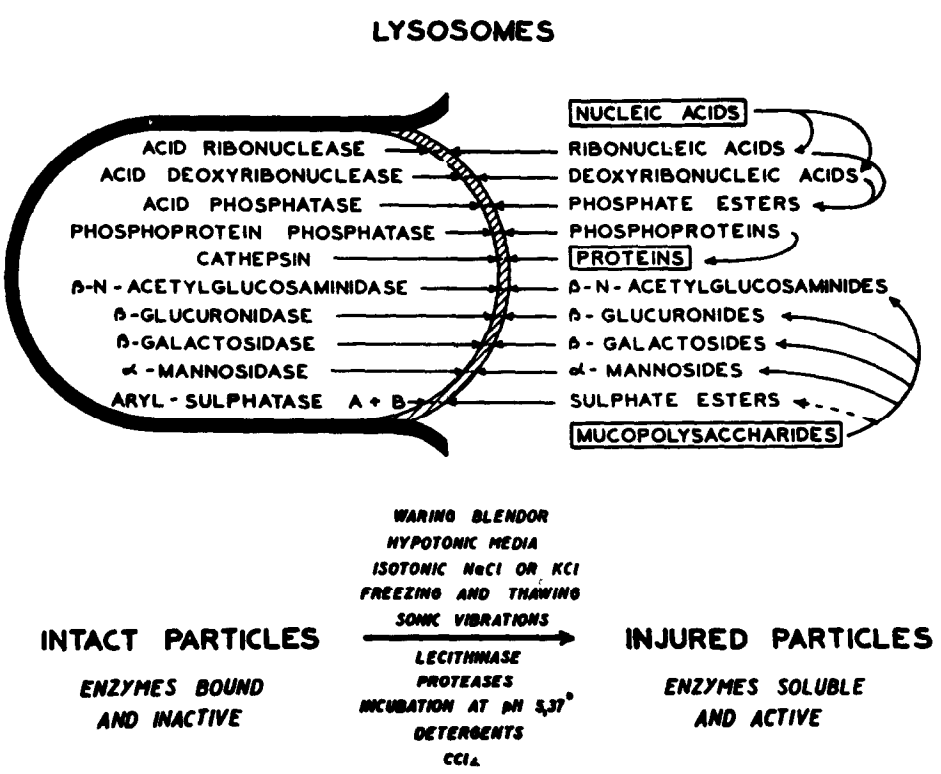

Fig. 1. Schematic representation of properties of rat-liver lysosomes.

Fig. 1 illustrates in schematic form the main properties of rat-liver lysosomes, as revealed by their behaviour *in vitro*. The essential characteristics are a sack-like structure with a mean diameter of about 0·4 μ, a surrounding membrane of lipoprotein nature and diffusible contents in which the various enzymes listed appear to be present in soluble form. The membrane acts as an effective barrier between the enzymes and their substrates, with the result that intact particles are practically inactive enzymically on external substrates. The various kinds of treatment listed in the diagram all cause the membrane to become injured, by a variety of mechanisms, and consequently bring about a partial or complete unmasking of the enzymic activities. This phenomenon is generally associated with a release of the enzymic proteins into the soluble phase, but there may be limiting conditions where penetration of substrates has become possible while the enzymes still are retained by the partially damaged membrane.

The picture of Fig. 1 is based entirely on biochemical observations, but is not inconsistent with morphological studies. It has been possible to show that the lysosomes of rat liver correspond to the peribiliary dense bodies (Fig. 2) which possess a single surrounding membrane and are seen to release acid phosphatase in sections treated with detergents or exposed to distilled water.

There is a fair amount of evidence that lysosomes are concerned with processes of intracellular digestion, especially in phagocytic cells, and are involved in various physiological and pathological phenomena of autolysis and necrosis. The way in which they are believed to participate in the latter phenomena is illustrated schematically in Fig. 3. It is essentially a transposition of the properties exhibited by the isolated particles to *in vivo* conditions. The particle membrane is assumed to protect the cell contents against digestion by the lysosomal enzymes, while injuries to this membrane would be expected to permit the diffusion of the hydrolytic enzymes into the surrounding cytoplasm and thus to bring about a greater or lesser degree of autolytic damage to the cell, leading in some cases to cell death and necrosis. Such a change has already been shown to occur in several tissues containing a large proportion of dead or dying cells, but it is not yet known with certainty whether it is a causal factor of cell death or takes place as a post-mortem phenomenon. All that can be said at the present time is that it occurs very early in cells exposed to unfavourable conditions and may therefore be the actual cause of cell death, at least in some cases.

This still partly hypothetical picture carries an important pharmacological implication that compounds which labilize the lysosomal membrane may stimulate autolytic processes and even cause widespread lesions in susceptible tissues, whereas those which strengthen the membrane against the stresses to which it may be normally or pathologically

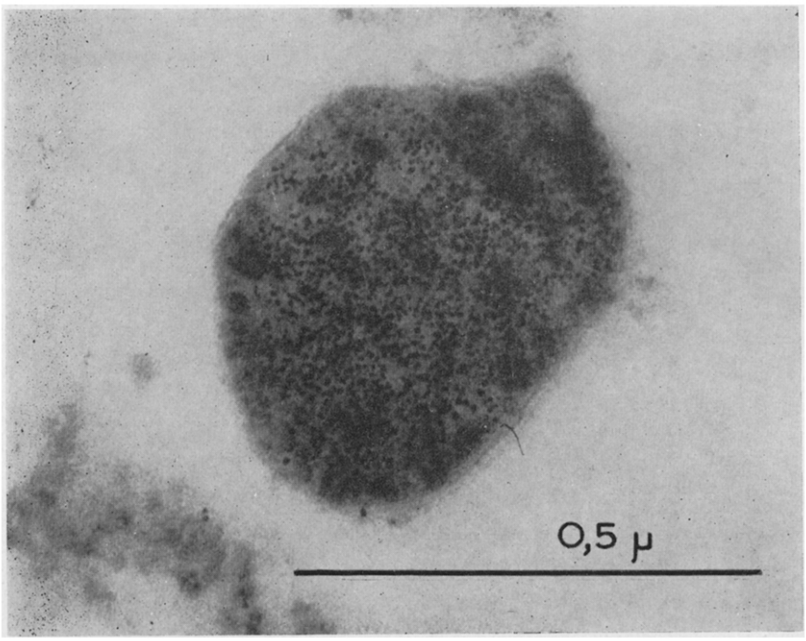

FIG. 2. Electron micrograph of a pericanalicular dense body (lysosome) from rat liver. Ultrathin section through a purified preparation isolated by density-gradient centrifugation.

exposed may prevent or retard autolysis and exert a protective effect on the cells under some conditions.

That such agents may be found preferentially, but not perhaps exclusively, amongst fat-soluble compounds was suggested by the lipoprotein nature of the lysosomal membrane and also by a few recent observations. For instance, it has been found by Dingle (1961) that

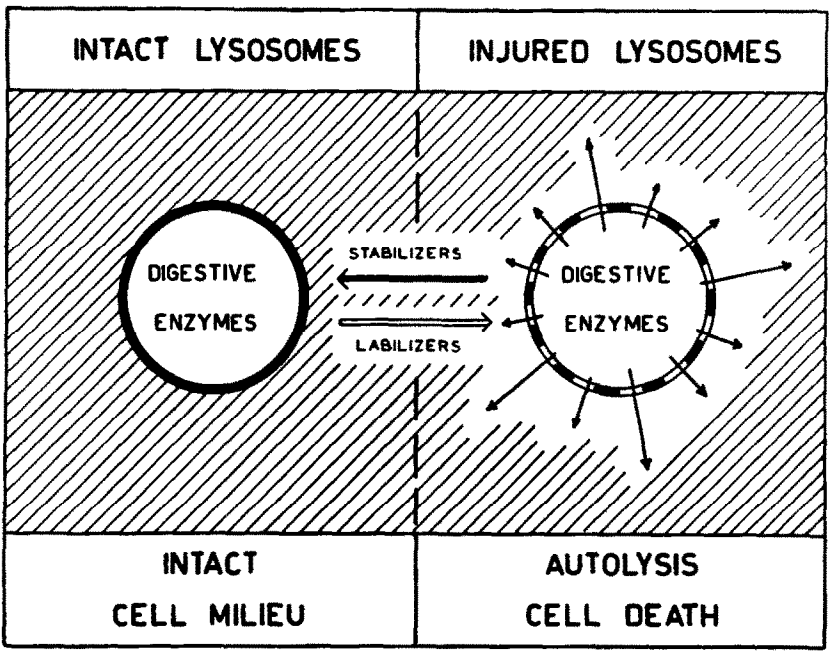

FIG. 3. Schematic view of autolytic role of lysosomes and of possible drug actions on cells through lysosomes.

excess vitamin A causes the release of cathepsin from hepatic lysosomes *in vitro*, and the results obtained by Dingle *et al.*, (1961) and Lucy *et al.* (1961) on embryonic bone transplants indicate strongly that a similar phenomenon takes place in cartilage cells and that it is probably responsible for the toxicity of vitamin A. Fell and Thomas (1961) have found that hydrocortisone inhibits this effect of vitamin A and Weissman and Dingle (1961) have observed a similar protective action of hydrocortisone on rat-liver lysosomes against the disruptive effect of u.v. irradiation. In experiments from this laboratory, some evidence of decreased lysosomal rupture in the livers of rats treated with hydrocortisone has been obtained (Beaufay *et, al.*, 1959). A stabilizing influence of vitamin E on muscle lysosomes is suggested by the results of Tappel and co-workers, who have found signs of lysosomal damage in the muscles of vitamin E-deficient animals (Zalkin *et al.*, 1961; Tappel, private

communication). According to these authors, vitamin E may act by inhibiting lipid peroxidation in the lysosomal membrane (as well as in other membranous structures).

In view of these considerations, it was decided to make a survey of the effects of fat-soluble compounds of biological interest on the integrity of lysosomes exposed *in vitro* to unfavourable conditions of a type likely to occur *in vivo*. The present report summarizes the first results obtained in these experiments.

METHODS

Amongst the various treatments listed in Fig. 1 as causing damage to lysosomes, incubation at pH 5 and 37° in an osmotically satisfactory medium was chosen as reflecting most closely a possible *in vivo* situation. Necrosis is known to be associated with a lowering of the intracellular pH and acidification has been suspected of being the intracellular change responsible for the disruption of lysosomes which takes place in anoxic cells (de Duve and Beaufay, 1959). For reasons of technical facility, free acid phosphatase was measured routinely to assess the degree of lysosomal disruption, but in a number of cases the results were verified on other lysosomal hydrolases.

The experimental procedure was as follows. Mitochondrial fractions from the livers of male rats, corresponding to the sum of fractions M and L of de Duve *et al.* (1955) and containing the larger part of the lysosomes, were prepared in 0·25 M sucrose. One part of the particle suspension was mixed with one-hundredth its volume of a concentrated solution of the compound tested dissolved in dioxane or ethanol, while another part was mixed with the solvent alone to serve as control. After addition of an equal volume of 0·1 M acetate buffer pH 5 made up in 0·25 M sucrose, these suspensions were incubated at pH 5 and 37°. During this preliminary incubation, the concentration of acetate + acetic acid was 0·05 M, that of the solvent 0·5% (V/V) and that of the compound tested generally 0·125 mg/ml. Each ml. of suspension contained the particles from 0·125 g fresh liver, so that the ratio of compound to particles was 1 mg/g original liver, or approximately 0·02 mg/mg mitochondrial protein or 0·14 mg/μmole mitochondrial phospholipid. It should be remembered that mitochondria are the main component of the fractions used and that the lysosomes occur in the preparation essentially as contaminants. In some experiments, concentrations lower or higher than 0·125 mg/ml. were also used.

To measure free acid phosphatase, β-glycerophosphate was added to a final concentration of 0·05 M, as a solution in 0·25 M sucrose containing 0·05 M acetate buffer pH 5 in order not to modify the composition of the medium. The incubation was continued for another 10 min and the inorganic phosphate set free was determined by a colorimetric

procedure. Total acid phosphatase was measured in a similar manner with the addition of 0·1% Triton X-100 to disrupt intact particles.

Fig. 4 shows the course of release of acid phosphatase in control preparations incubated under the conditions described. As illustrated graphically in this figure, the release of enzyme presumably continues

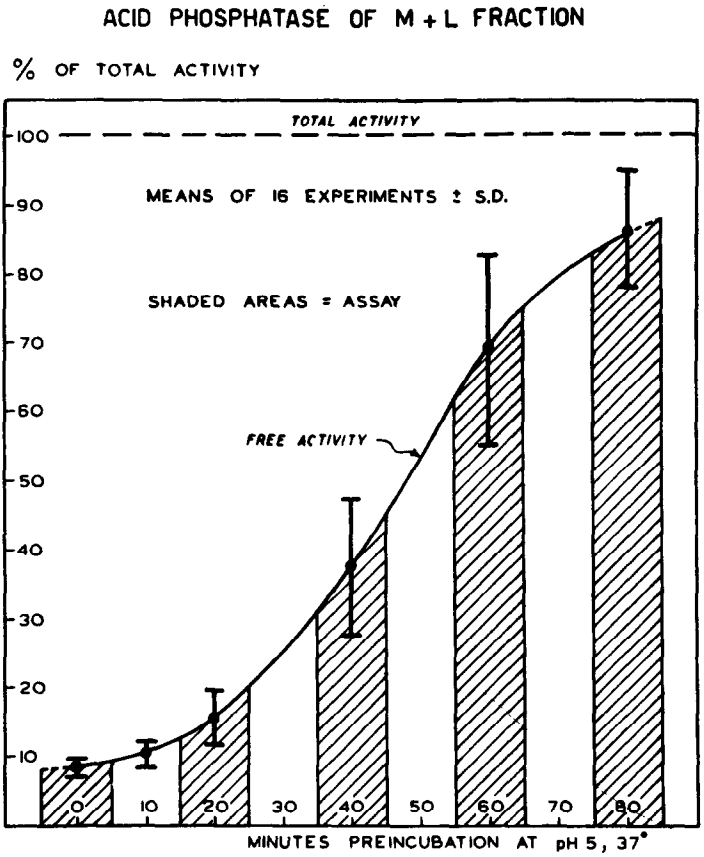

FIG. 4. Kinetics of release of acid phosphatase.

during the 10 min incubation necessary for the assay and the activities observed may be taken to reflect as a first approximation the state of the lysosomes after $x + 5$ min at pH 5 and 37°, x being the preincubation time. In many experiments, complete curves of the type shown in Fig. 4 were run in the presence and absence of the sample tested. In others, measurements were made only before and after preincubation for a given length of time, usually 40 min. In some fifty preparations studied so far, the free activity rose from an initial value of about 9% to some 40% of the total activity after 40 min. Individual preparations varied quite widely in their rate of disruption, as is also shown by the large standard deviations in Fig. 4. When necessary for the purpose

of comparison or of statistical evaluation, the results obtained on different preparations were recalculated in terms of a hypothetical standard control showing 10% initial free activity and 40% free activity after 40 min.

RESULTS

1. Solvents and Digitonin

At the concentration used in these experiments (0·5%) ethanol and dioxane usually show a slight accelerating effect on the rate of disruption of the lysosomes at pH 5 and 37° (see for instance: Figs. 6 and 7). This effect becomes very pronounced with 5 or more times the usual concentration. However, even at a 5% concentration, the solvents were never found to affect significantly the initial free activity of the preparations, which indicates that their influence on the particle mem-

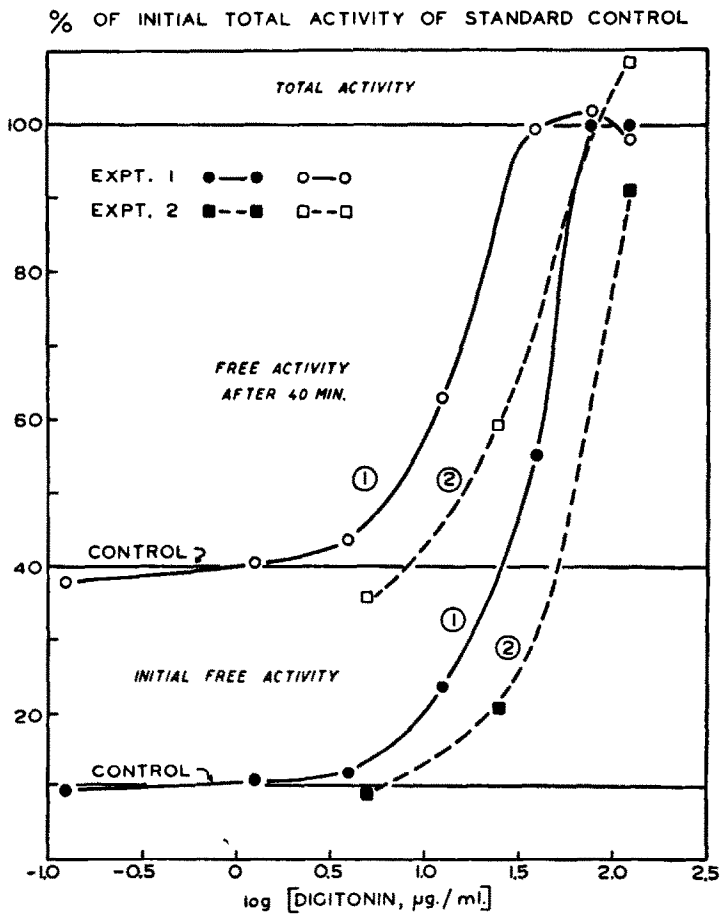

FIG. 5. Effect of digitonin. Results of 2 experiments. Solid symbols: initial free activity; open symbols: free activity after 40 min preincubation at pH 5 and 37°.

brane is not an immediate one and hardly manifests itself during the first 10 min of incubation at pH 5 and 37°.

Such is not the case with digitonin. As shown by Fig. 5, this compound exerts a strong disruptive effect on lysosomes and brings about an essentially complete release of acid phosphatase under our usual test conditions. In one experiment, a dose as low as 40 μg/ml was sufficient to cause this effect. At lower doses, the influence of digitonin decreases and it becomes negligible at a concentration between 2 and 10 μg/ml. One important feature of digitonin, clearly illustrated in Fig. 5, is that any concentration which affects the rate of release of acid phosphatase at pH 5 and 37° (effect at 40 min) also affects the initial free activity (effect during the first 10 min). It is relatively unique in this respect.

2. *Vitamins and Related Compounds*

A panoramic view of the results obtained in the standard test system with fat-soluble vitamins and various structurally related compounds is given below in Fig. 10. While confirming the effect of vitamin A on lysosomes, first observed under different conditions by Dingle (1961), our experiments have shown that vitamins D_2, E and K_1, as well as the related substances phytol and ubichromenol, also promote the release of the lysosomal acid phosphatase at pH 5 and 37°. On the other hand, cholesterol was found to have a distinct inhibitory influence on this phenomenon. Some of the effects observed were studied in greater detail.

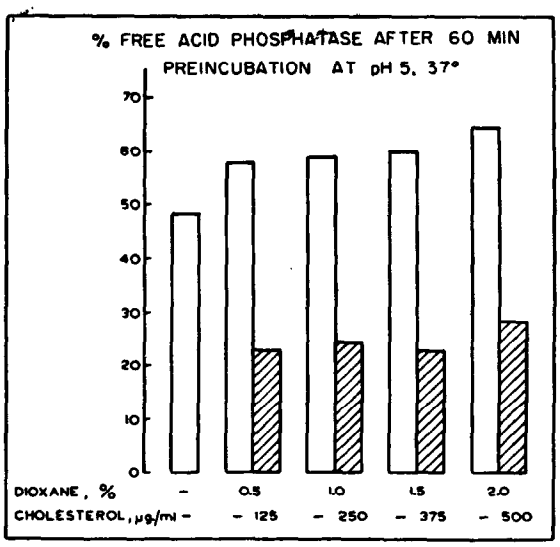

FIG. 6. Effect of cholesterol. Results observed after 60 min preincubation in preparations mixed with increasing amounts of a 25 mg/ml solution of cholesterol in dioxane or of dioxane alone.

a. *Cholesterol and Vitamin D* — As illustrated by Figs. 6 and 7, the stabilizing effect of cholesterol on the lysosomes can be very striking in some cases and result in a marked protection of the particles, even in the presence of relatively large amounts of dioxane. Fig. 7 also shows that cholesterol has to be added in solution to be effective. When it was added as a suspension made in 0·25 M sucrose with the help of a Potter-Elvehjem homogenizer, no effect of the sterol on the release of acid phosphatase could be observed. In one experiment, decreasing the dose to 25 μg/ml. or increasing it to 625 μg/ml. resulted in only a slight change in the degree of protection of the lysosomes. So far, the minimum effective dose of cholesterol has not yet been determined. Neither has it been ascertained whether cholesterol can protect the lysosomes against the disruptive effect of some of the steroids and other compounds investigated in this study.

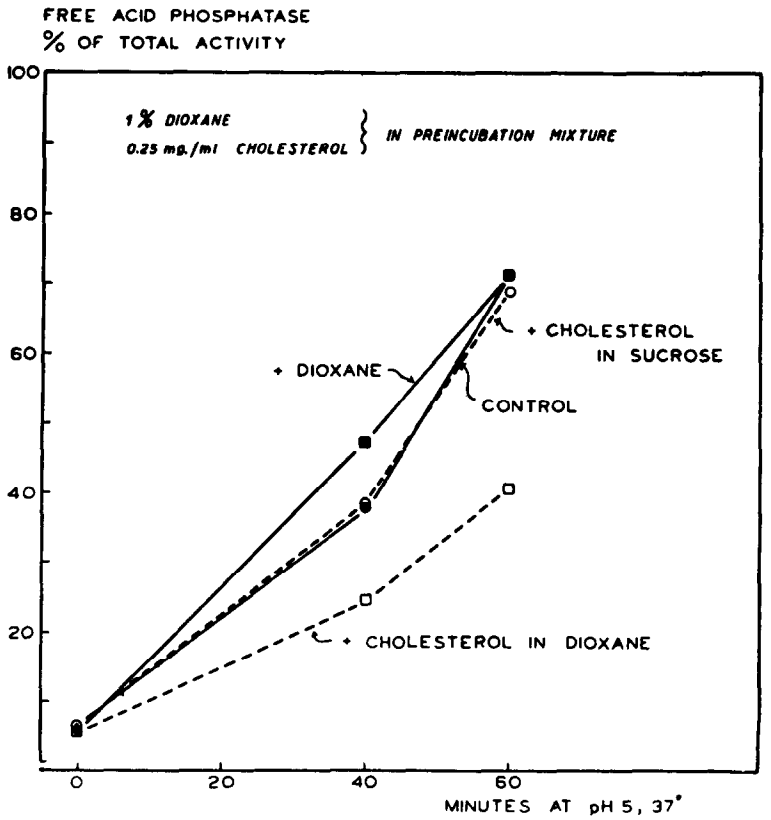

FIG. 7. Effect of cholesterol. Solid symbols: controls; open symbols: + cholesterol. Squares: cholesterol added as a 25 mg/ml. solution in dioxane; dioxane added in control. Circles: cholesterol added as 25 mg/ml. suspension in 0·25 M sucrose, dispersed in a Potter-Elvehjem homogenizer; sucrose in control.

It is interesting to note that vitamin D_2 not only failed to show the effect of cholesterol but markedly increased the rate of disruption of the lysosomes (see Fig. 10).

b. Vitamin A — According to Dingle (1961), vitamin A stimulates the thermal release of soluble proteolytic activity from the lysosomes at pH 7, but not at pH 5. In the present experiments, it was found that vitamin A promotes the appearance of free acid phosphatase both at pH 5 and at pH 7. Dingle's failure to observe an effect at pH 5 is probably due to some kind of adsorption artifact, for it has been found in preliminary experiments that cathepsin is strongly retained by the particulate components of the preparation at pH 5, but not at pH 7, even after it has become active towards an external substrate. In confirmation of observations made by Dingle (personal communication) on cathepsin, vitamin A palmitate (Fig. 10) or acetate showed little or none of the disruptive activity of the free alcohol.

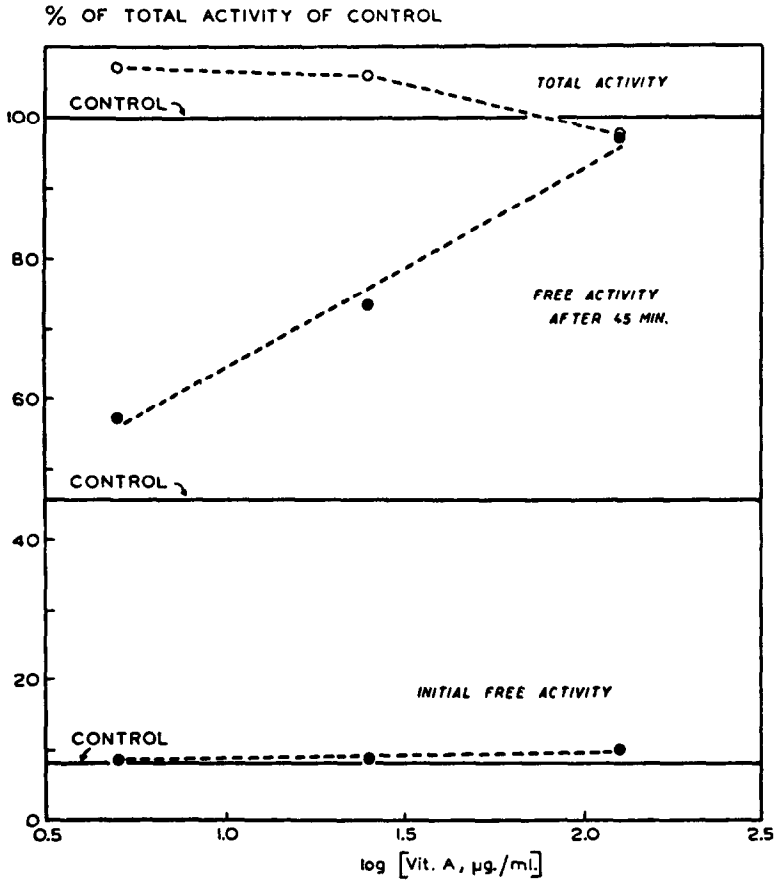

FIG. 8. Effect of vitamin A. Solid circles: free activities before and after 45 min preincubation at pH 5, 37°. Open circles: initial total activities.

In Fig. 8 are shown the results of an experiment in which the influence of vitamin A concentration was investigated. The vitamin appears to be almost as active as digitonin in preparations preincubated for 40 min and may even be more active at low concentration (see Fig. 5). However, in contrast with the glycoside, it is practically without effect on the initial free acid phosphatase activity, even at the highest concentration tested.

c. Vitamins E and K and Related Compounds — According to the results represented graphically in Fig. 9, vitamin E appears to be slightly less active than vitamin A on a weight basis, which makes it about equally active on a molecular basis. With a single exception, vitamin E

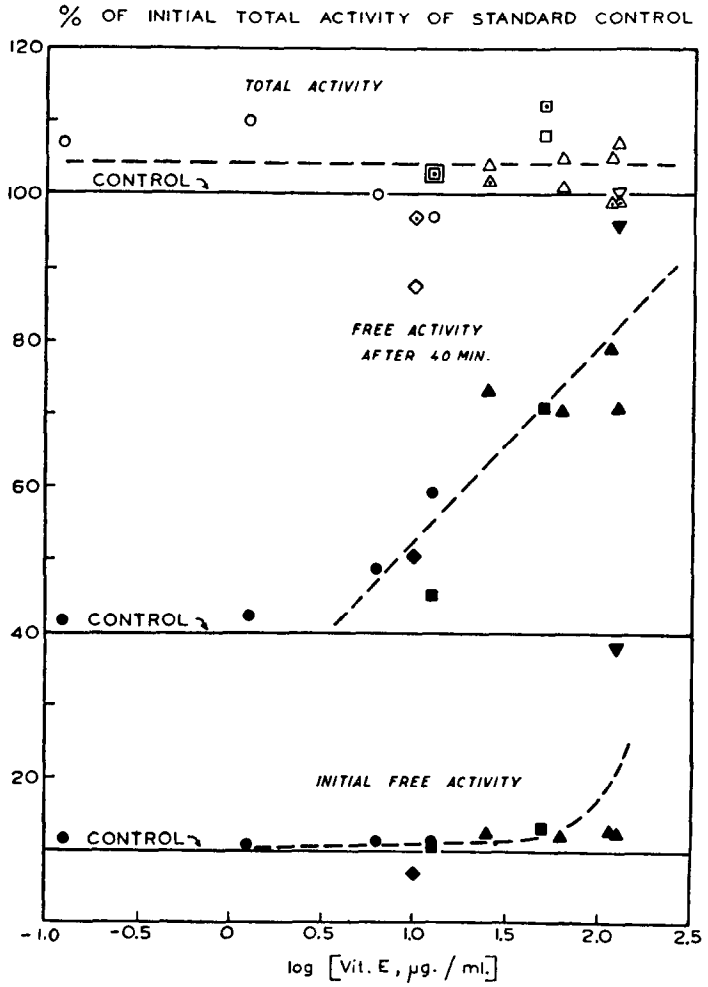

FIG. 9. Effect of vitamin E. Results of 5 experiments, represented by different signs. Solid symbols: free activities before and after 40 min preincubation at pH 5, 37°. Open symbols: total activities before and (with central point) after preincubation.

had little effect on the initial free activity, as was found with vitamin A. In contrast with the negative results obtained with vitamin A esters, vitamin E acetate also showed considerable activity in one experiment.

Thanks to the courtesy of Dr. Karl Folkers from the Merck Therapeutic Research Institute, ubiquinone (Co Q_{10}) and its reduction product, DL-ubichromenol, could also be tested in this system. Ubichromenol, which bears several structural relationships to vitamin E, was found to be quite active in releasing acid phosphatase and could be as effective as vitamin E on a molecular basis. Direct comparison of the compounds

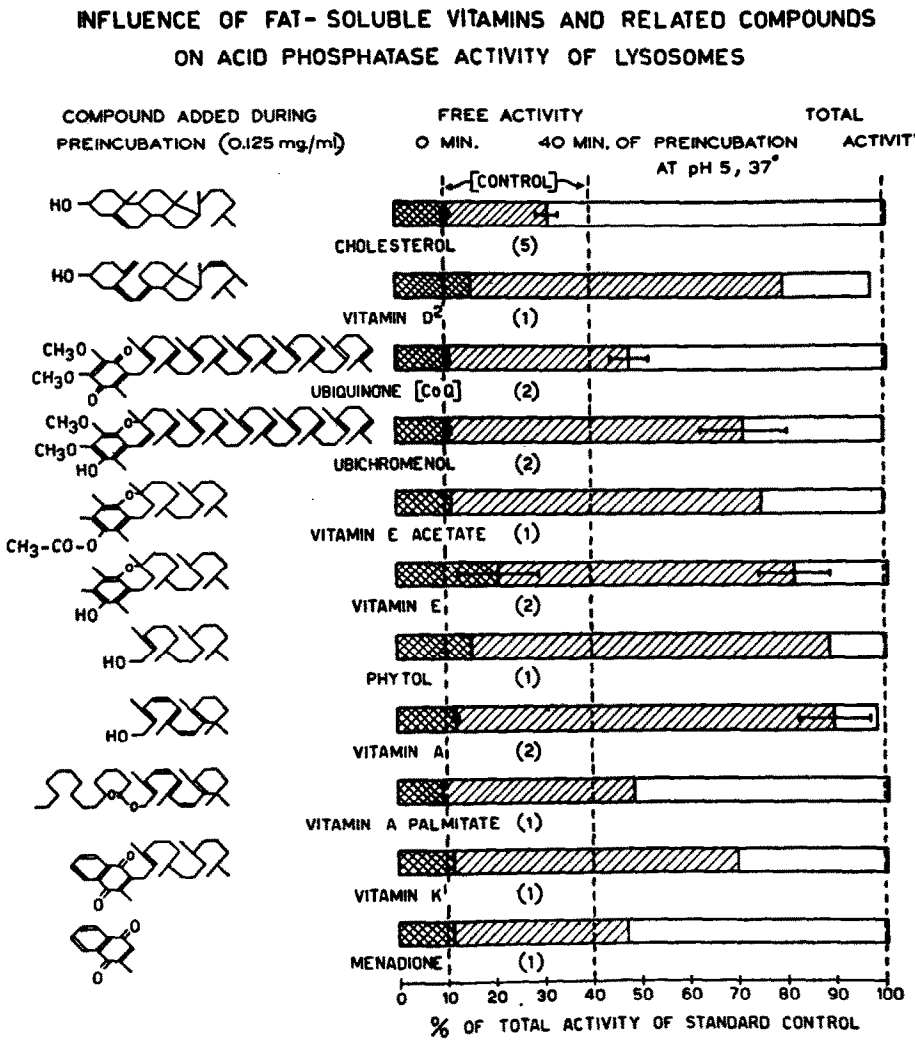

FIG. 10. Effect of fat-soluble vitamins and related compounds. Between brackets: number of experiments. Results are given as means ± S. E. M. Total activities are average of initial and final values.

was not made. On the other hand, in two experiments performed on the same preparations with the two coenzyme Q derivatives, ubiquinone proved distinctly less active than ubichromenol (Fig. 10).

In one experiment, vitamin K_1 and its two components, phytol and menadione, were tested on the same preparation. As shown by Fig. 10, the naphthoquinone part of the molecule had little activity by itself, whereas phytol accelerated very markedly the release of acid phosphatase.

d. Structural Relationships — The results obtained so far are too scanty to allow a detailed examination of the chemical structural factors involved in the observed effects, but some preliminary deductions can be drawn.

From a consideration of Fig. 10, it appears that the most active compounds have in common a poly-isoprenoid chain and a free hydroxyl end-group. The length of the side-chain, its degree of unsaturation and the introduction of partial rigidity by cyclization do not seem to influence greatly the effectiveness of the compounds in promoting the disruption of lysosomes, as is indicated by comparison of the effects of phytol, vitamin A, vitamin E, ubichromenol and vitamin D_2. However, sufficient coiling freedom is probably essential, as is shown by the drastic reversal of activity from vitamin D_2 to cholesterol, which is most likely to be due to the closing of ring B. Oxidation of the terminal hydroxyl seems to reduce the activity, while its esterification largely suppresses it in the case of vitamin A, though not, apparently, in that of vitamin E. This discrepancy requires further investigation.

3. *Steroids*

a. Sex Hormones — Three hormonally active compounds, testosterone propionate, β-oestradiol benzoate and progesterone, were found to accelerate markedly the release of acid phosphatase. The results obtained after 40 min are shown in Fig. 11 and 12, but the effects were verified after shorter and longer periods of incubation. As with most other active compounds, no influence was seen on the initial free activity.

A possible relationship with hormonal activity is suggested by the fact that the relatively weak oestrogen oestrone proved much less active than oestradiol, whereas pregnandiol and various other progesterone derivatives had little or no activity (see below). However, a great many more experiments are required before any definite conclusion can be drawn on this point.

b. Corticoids — The effects observed with various naturally occurring corticosteroids are represented in Fig. 72. In order to allow an examination of the structural relationships involved, this figure also includes the results obtained with progesterone, pregnandiol and a number of

synthetic pregnane derivatives kindly furnished by Dr. A. E. Heming from the Smith, Kline and French Laboratories.

With the natural steroids, there is a progressive gradation from deoxycorticosterone, which still shows part of the disruptive activity of

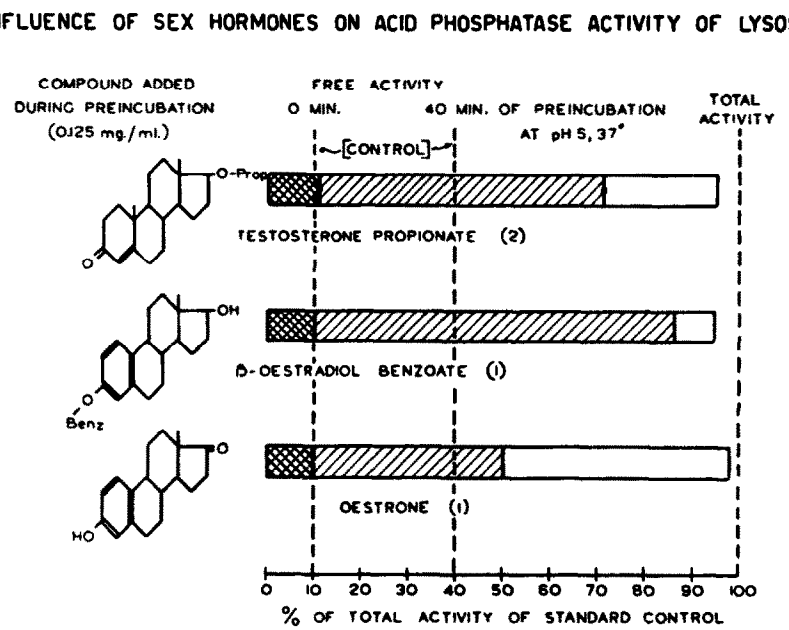

FIG. 11. Effect of sex hormones. Between brackets: number of experiments. Total activities are average of initial and final values.

progesterone, through the slightly effective corticosterone and the essentially inactive deoxycortisol (Reichstein compound S) to cortisol and cortisone, which inhibit the release of acid phosphatase. In one experiment, this gradation was verified on the same particle preparation. In general, the synthetic steroids showed less activity than their closest natural parent compound. In one experiment with aldosterone, a strong inhibition of the release of acid phosphatase was observed after 40 and 60 min preincubation. In another experiment, however, no effect was found after 40 min.

The effect of cortisone was further studied in a number of experiments. Though less marked than that of cholesterol, it has been regularly observed and proved relatively important in some cases (Fig. 13). It was also found in preparations treated with testosterone, deoxycorticosterone or vitamin E, but resulted only in a partial inhibition of the disruptive effect of these compounds. In one experiment, concentrations of 25, 125 and 375 μg/ml inhibited the release of acid phosphatase to about the same degree. Cortisone acetate proved as effective as the free alcohol (Fig. 12) and a similar activity was also observed in single experiments

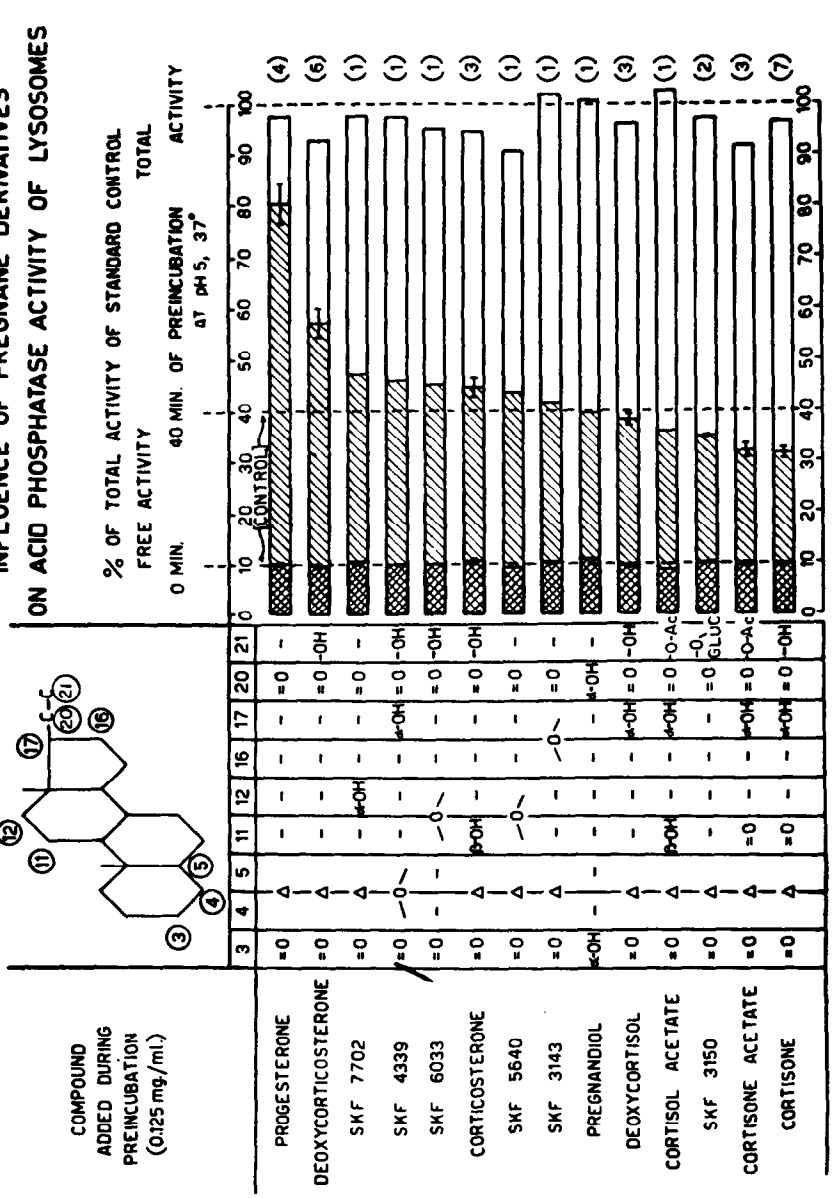

FIG. 12. Effect of pregnane derivatives. Between brackets: number of experiments. Results are given as means ± S. E. M. Total activities are average of initial and final values.

with cortisol acetate and with dexamethasone (1-dehydro-16α-methyl-9α-fluorohydrocortisone). The latter was however much less active than cortisone (Fig. 13). One synthetic steroid (SKF 3150), the 21-O-glucoside of deoxycorticosterone also inhibited the release of acid phosphatase. According to a private communication from Dr. A. E. Heming, this compound is a potent salt-retaining substance *in vivo* and is as effective as deoxycorticosterone in raising the electroshock threshold. This would indicate a lack of correlation between biological property and activity in the present test system, unless rapid deglucosidation occurs *in vivo*.

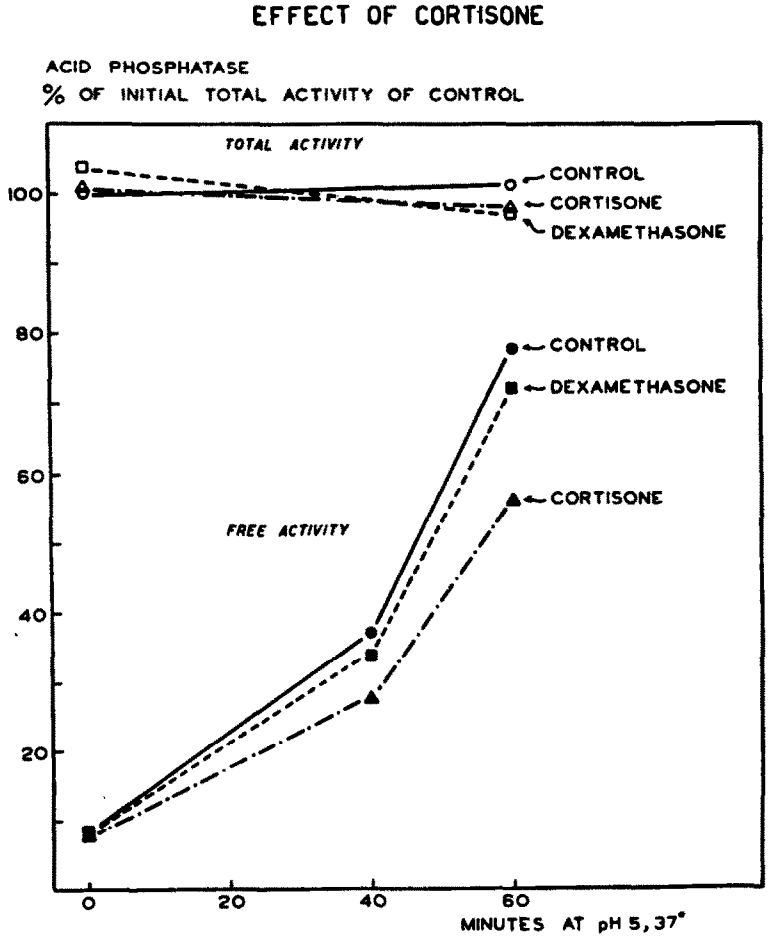

FIG. 13. Effect of cortisone and dexamethasone.

There is one difficulty which complicates the interpretation of the results observed with cortisone. This compound has been found to cause a significant decrease of the total phosphatase activity in the course of preincubation. In 7 experiments, the total activity was found to fall from a mean value of 99·3 ± 1·5 to 93·5 ± 2·5% after 40 min. The

nature of this effect is not known, but if it were due to a selective inactivation of the free acid phosphatase, it could account almost entirely for the decrease in free acid phosphatase activity as observed in many experiments, though not, however, in all (see for instance Fig. 13).

c. Structural Relationships — The relationship between chemical structure and activity in the acid phosphatase test system is shown in Fig. 12. In general it may be said that the introduction in the pregnane molecule of an increasing number of oxygen atoms, especially in hydroxyl form, tends to decrease and then reverse the disruptive action of the resulting steroid. Too few compounds with androgenic or oestrogenic activity have been assayed to appreciate the role of the side-chain and of the unsaturation of the A ring.

DISCUSSION

1. *Mechanism and Chemical Specificity of Observed Effects*

Little is known concerning the nature of the phenomena involved in the disruption of lysosomes as it occurs under the conditions of the present assays. The process has been studied by Berthet *et al.* (1951), Appelmans and de Duve (1955) and Beaufay and de Duve (1959), and it has been suggested, on the basis of kinetics, temperature coefficient and pH dependence, that it may involve an enzymic reaction, possibly a proteolytic attack of the lysosomal membrane by the particles' own cathepsin. Even if such a mechanism is implicated, it is likely that it serves only to initiate a sequence of events, which may include changes in permeability, diffusion of solutes and an osmotic type of swelling, before leading to the final disruption of the lysosomes.

Obviously, the rate of such a complex process may depend on a number of variables and the effects brought to light in the present investigations may be due to a variety of mechanisms. It is possible, for instance, that the compounds tested act by affecting the activity of the enzyme systems assumed to be involved in the disruption of the lysosomes. Since the nature of these systems is unknown, this possibility is not open to direct verification. An effect of vitamin A on the activity of the lysosomal cathepsin has, however, been ruled out by Dingle (1961).

Another hypothesis is that the compounds tested interact with the membrane of the particles and either influence its susceptibility to the attacking enzymes or modify the way in which its physical properties are affected by a given degree of enzymic degradation. This possibility is directly supported by the observation that cholesterol exerts a distinct protective effect on the disruption of lysosomes, as studied in these experiments. Cholesterol is known to be an important constituent of biological membranes and is believed on several grounds to act as

stabilizer of the phospholipid layer in these membranes (Winkler and Bungenberg de Jong, 1941; Finean, 1953; Willmer, 1960). The present results are consistent with a similar interpretation and suggest that cholesterol is also a stabilizer of the lysosomal membrane.

It has been proposed by Willmer (1960) that biologically active steroids might exert their effects by replacing cholesterol molecules at certain sites of the membranes and thus modifying the structure and physical properties of the latter. This theory could possibly be extended to other lipid-soluble components, such as the vitamins and other derivatives used in the present investigations. It is, however, not easy on the basis of the data presently available, to present a valid interpretation of the nature of the phenomena involved.

As already pointed out above, the stabilizing effect of cholesterol seems to be partially retained in steroids which are richest in polar groups, especially hydroxyl groups. As these are lost, stabilization disappears and is replaced by a labilizing influence which is strongest with progesterone, and also, for reasons which are not immediately apparent, with the sex hormones. Another factor which may be of importance for the stabilizing effect is the rigidity of the molecule. It is very striking that a strong disruptive effect was observed with vitamin D_2, which probably owes this property mainly to the opening of the B ring. Most of the isoprenoid derivatives which exert a similar effect have in common with vitamin D_2 a polar end-group and a relatively flexible chain.

Whichever the actual mechanisms involved, it is clear that small chemical changes may affect greatly the nature of the effect exerted by lipid-soluble compounds on the lysosomal membrane. As such, these compounds provide a delicate tool whereby the properties of different biological membranes may be explored and compared with each other.

In experiments performed with comparable concentrations of active compound, Viala and Gianetto (1957) have observed no effect of cholesterol, nor of progesterone, deoxycorticosterone acetate or testosterone, on the osmotic resistance of lysosomes, but noted a certain degree of protection against osmotic lysis with oestradiol benzoate and testosterone propionate. Direct comparison of their results with those reported here is difficult, since they exposed their preparations to the agent tested for only 15 min at 0°, whereas the effects observed in the present experiments generally become evident after a longer time of contact at pH 5 and 37°. Digitonin is exceptional in this respect and appears to act instantaneously. Presumably, it has a different mode of action, depending either on its high surface activity or, possibly, on the removal of cholesterol molecules from the membrane in the form of the insoluble digitonide.

2. *Biological Significance of Observed Effects*

It is difficult to decide at the present time whether the effects observed in these experiments bear any relation to the biological properties of the compounds tested. The best documented case is that of vitamin A, whose effects *in vitro* have been well correlated with its *in vivo* properties in the investigations of Dingle, Fell and co-workers quoted in the introduction to this paper. As pointed out in the introduction, there is also some evidence that the protective influence of cortisone and hydrocortisone on the lysosomes is of physiological significance and it is tempting to speculate on the possibility that it may be related to the anti-inflammatory properties of these steroids. On the other hand, it is surprising that dexamethasone, which is a potent cortisone analogue, was relatively ineffective as a lysosome protector, whereas SKF 3150, which has deoxycorticosterone-like properties, was. It must also be pointed out that the *in vitro* effect of vitamin E does not appear to be reflected in an *in vivo* toxicity analogous to that of vitamin A. On the contrary, the work of Tappel and co-workers quoted above suggests that vitamin E has a protective action on the lysosomes, at least of muscle tissue, in the whole animal. It is also obvious that the actions observed cannot possibly account solely for the variety of biological effects exerted by the fat-soluble vitamins and steroids nor for the specificity of these effects. Finally, the doses necessary to obtain an *in vitro* effect of the biologically active compounds exceed by far those which suffice to cause a response in the living animal. In view of these reasons, it might appear likely that, with a few possible exceptions, the results obtained in the present investigations are of little physiological or pharmacological interest.

However, one should not be too categorical in this respect. The procedure used in these experiments does not permit any evaluation of what was in fact the concentration of the active compounds, especially since most of them are essentially insoluble in water, and the conditions of incubation are so far removed from those which obtain in living cells as to make a comparison in terms of concentration extremely hazardous. It must also be remembered that our test system involves a single organelle from a single tissue. It is quite possible that the compounds studied may act differently on the lysosomes of different tissues and it is also likely that they will act on other structures such as the cell membrane, the mitochondria or the endoplasmic reticulum. In recent years, several effects of steroids on mitochondria have been described (Blecher and White, 1960; Dietrich *et al.*, 1961; Gallagher, 1958, 1960) and these have generally required the same high concentrations as were used in the present experiments.

One could therefore put forward the alternative view that the observed effects are not without biological significance but must be

interpreted within the framework of a broader concept of membrane architecture and function. In recent years, the electron microscope has revealed the abundance and complexity of membranous structures in living cells, while biochemical and biological studies have made it clear that these membranes are not simply inert barriers, but are the site of many of the most intricate processes of living cells. A great deal of evidence has been adduced in support of the theory that membranes are made of a lipoid sheet, probably formed by a double monolayer of phospholipids joined by their non-polar groups and projecting their polar groups outward, on which are attached various proteins and carbohydrate components. It is, however, obvious in view of the multiplicity and specificity of the functions carried out by the various membranes, that this picture provides only a basic pattern, of which there must be innumerable variants, differing by the nature and mode of association of the catalysts included in the structure, as well as by the arrangement of the lipid constituents. As pointed out by Willmer (1961), the latter provide a natural medium in which steroids and other fat-soluble compounds may be expected to accumulate and *in vitro* studies have shown that such compounds can be incorporated within the texture of lipid films. It is therefore not unreasonable to think of the lipid part of biological membranes as having a *mosaic* type of structure with specific sites or *holes* occupied by sterols, steroids, terpenes and various fat-soluble catalysts, and to assume that the nature and abundance of these components may affect in a specific manner the physico-chemical properties of the areas which they occupy. How such influences will be reflected at the biological level will depend on the functions carried out by the particular area or membrane concerned.

The effects observed in the present studies, although they may not all be of equal significance or importance, could be fitted in a general view of this type. Their particular interest resides in the fact that they exemplify the possibility of a novel type of hormonal or pharmacological action, exerted not on the actual activity of enzyme systems but on their topographical distribution within the cell.

SUMMARY

Various fat-soluble compounds of biological interest have been added, generally at a concentration of 0·125 mg/ml., to mitochondrial fractions from rat liver incubated at pH 5 and 37° in 0·25 M sucrose, and the rate at which acid phosphatase is released from the lysosomes has been followed. Considerable acceleration of this process was observed in the presence of digitonin, vitamins A, D_2, E and K_1, ubichromenol, phytol, testosterone propionate, β-oestradiol benzoate and progesterone. Cholesterol, cortisone and hydrocortisone were found to have a retarding effect on the release of acid phosphatase. Other compounds

chemically related to the above exerted relatively small effects or were without influence on the phenomenon studied. Some of the chemical factors involved in the observed effects have been identified and the biological significance of the results has been discussed.

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