progeny averages (Table, b and c) were slightly lower than predicted, but it is likely that some trapping of viable embryos occurred in oothecae counted as 'productive'. This could also account for the average of 6.8 offspring in d, in which crosses between interchanges with random disjunction were expected to average ca. 9–10 nymphs/ootheca. Also this for reason, it seems likely the lethality estimate of 82% is somewhat high. The lethality expected from crosses between 2 translocations with random disjunction is 75%.

We cannot yet account for the lack of productivity and apparent high lethality in crosses of the double heterozygote of T(2;11)*Cu*; T(9;?) (Table, e). The data suggest an unexpectedly low production of balanced gametes although, in the single stock of T(9;?), alternate disjunction is favored in the males. From the data presented it appears that disjunction and lethality in the T(3;12) double could be predicted on the basis of the characteristics of the single stocks, but this is not true for the T(9;?) crosses.

From the standpoint of genetic control, the most important estimate is the average replacement per pair based on the total parental group (Table, col. 7). It appears that the maximum effect possible from initial releases of T(2;11)*Cu*; T(3;12) males (c) would be a significant retardation of population growth. Alternatively, the double heterozygote T(2;11)*Cu*; T(9;?) (e) could cause nearly complete population suppression. Both the higher lethality and sterility from embryonic trapping would enhanced the effects of introducting such double heterozygous stocks over those using single translocations, and may thereby partially overcome the lack of fit translocation homozygotes. The effectiveness of this approach would vary according to the particular stocks involved. From the present data, it appears that population Suppression could be achieved by using stocks combining two interchanges with little or no directed disjunction in males, provided they prove to be competitive.

A second type of combination from which unique advantages might be derived is the double heterozygote of translocations having one chromosome in common (3-chromosome doubles). Viable gametes formed following disjunction in the ring-of-six configurations would carry one or the other parental interchange[10]. Thus, matings would be similar to those of translocation homozygotes in that all offspring would be heterozygotes, although the latter would be divided between the 2 parental types. Our first attempt to develop a 3-chromosome double, using T(2;11)*Cu* with T(9;?), failed due to chromosome misidentification. Nevertheless, the principle is valid and stocks are on hand which can be used to test it[11].

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Differential Effects of Lipids on the Osmotic Fragility of Lysosomes

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Summary. The effect of a wide range of concentrations of oleic acid, oleyl alcohol and oleic acid methyl ester on lysosomal stability has been studied under both hypotonic and isoosmotic medium conditions. Both oleic acid and oleyl alcohol exhibited a biphasic interaction pattern with lysosomes; stabilizing at low concentrations and labilizing at high concentrations. Lysosome labilization by the ester required an initial lag period.

Membrane protection by lipid-soluble drugs has been observed in erythrocytes, lysosomes and catecholamine granules[2–7]. The study of lysosomal stabilization by anti-inflammatory drugs is of special interest, since lysosomal labilization and leakage of lysosomal enzymes are implicated as important factors in many inflammatory conditions. Assessment of lysosomal stabilization by drugs has usually been carried out under isoosmotic medium conditions. In order to have a meaningful degree of damage to lysosomal latency in control lysosomal suspensions, the suspensions had to be exposed to unfavourable conditions, i.e. prolonged incubation at 37 °C or 45 °C[6–8] or acid pH[5–7]. Under these conditions, extensive damage to membrane structural components might be brought about as a result of hydrolysis by the lysosomal enzyme composite.

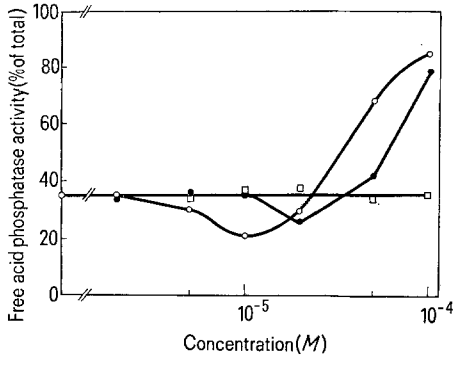

Fig. 1. Effect of oleic acid (○—○), oleyl alcohol (●—●) and oleic acid methyl ester (□—□) on the release of acid phosphatase from a lysosome-rich suspension. Lysosomes were incubated in 0.1 *M* sucrose, 0.01 *M* Na-Hepes buffer, pH 7, for 15 min at 37 °C.

[1] Acknowledgment. We should like to thank Mr. David Ginzburg for the assistance he gave us during the 3 months he spent at our department as a summer student.

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In the present study, we have adopted the system of exposure of lysosomal suspensions to the test compounds under mild hypotonic conditions. RAZ and LIVNE[9] have studied the differential effect of C_{18}-fatty acids, alcohols and methyl esters on the stabilization of erythrocytes against hypotonic lysis. It was of interest to assess the degree of parallelism that can be drawn between this system and the lysosomal system.

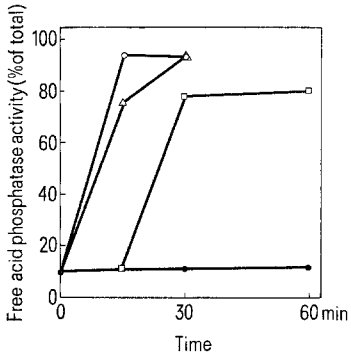

Fig. 2. Time course of acid phosphatase release from lysosomes upon exposure to 10^{-4} *M* oleic acid (○—○), oleyl alcohol (△—△) and oleic acid methyl ester (□—□). Control (●—●), 1% methanol in 0.25 *M* buffered sucrose. Incubation was carried out at 37 °C.

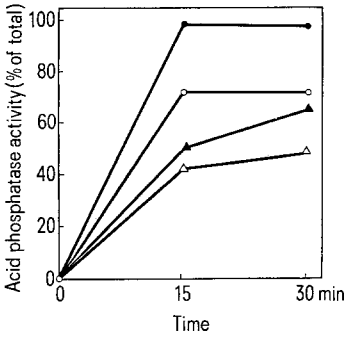

Fig. 3. Effect of 10^{-4} *M* oleic acid on free and available acid phosphatase activity in a lysosome-rich suspension. Lysosomes were suspended in either 0.25 *M* buffered sucrose (circles) or 0.25 *M* polyethylene glycol (triangles). (○—○) and (△—△) denote free activity, (●—●) and (▲—▲) available activity. Lysosomal suspensions were incubated for 15 min at 37 °C.

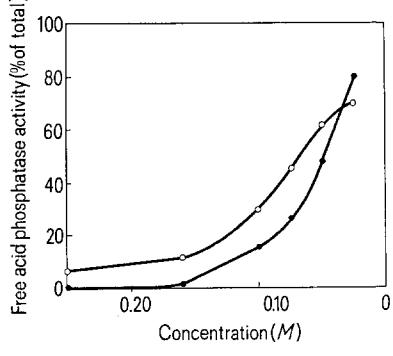

Fig. 4. The osmotic properties of lysosomes suspended in sucrose (○—○) or in polyethylene glycol (●—●). Suspending media were buffered with 0.01 *M* Na-Hepes, pH 7. Incubation was carried out for 15 min at 37 °C.

Materials and methods. Male CR strain rats (100–300 g) were starved for 16–24 h and killed by skull fracture. Livers were excised and rinsed with ice cold 0.45 *M* sucrose, 0.5 m*M* in EDTA, pH 7.2. The livers were minced, homogenized and subjected to differential centrifugation as described[10]. Stock lysosomal suspensions consisted of 24 mg protein/ml.

The effect of the test compounds, oleic acid, oleyl alcohol and oleic acid methyl ester (Sigma), on the latency of acid phosphatase, a lysosomal marker enzyme, was assessed using *p*-nitrophenyl phosphate as substrate. Aliquots (20 µl) from stock lysosomal suspensions were diluted into 1 ml of 0.1 *M* or 0.25 *M* of sucrose buffered with 10 m*M* of Na-Hepes, pH 7 (Sigma) containing either 10 µl of methanol or 10 µl of the test compound dissolved in methanol. After an incubation period (specified in the legends), total acid phosphatase activity in aliquots of the suspension, as well as non-particulate activity released to the medium, were measured as described[10,11]. Each experimental condition was repeated 3–6 times with different lysosomal preparations.

Results and discussion. The effect of various concentrations of oleic acid, oleyl alcohol and oleic acid methyl ester on the leakage of lysosomal acid phosphatase to the medium is given in Figure 1. Under the hypotonic conditions used (0.1 *M* sucrose), about 35% of total lysosomal acid phosphatase was released into the suspending medium. At a concentration of 10^{-5} *M*, oleic acid decreased this value to about 20%, while both the alcohol and the ester had have no stabilizing effect. At 2×10^{-5} *M*, oleyl alcohol exhibited a significant stabilizing effect, acid phosphatase leakage was reduced to a value of 25%. At a concentration of 5×10^{-5} *M*, both the acid and the alcohol induced extensive acid phosphatase leakage to the medium. It is of interest that the ester did not exhibit either the stabilizing or the labilizing effect at the entire range of concentrations that have been tested.

The pattern and the concentration dependence of the biphasic effect of oleic acid and oleyl alcohol conform to those observed in erythrocytes[9] and in liposomes[12]. The higher stabilizing potency of the acid over that of the alcohol was even more dramatic in the erythrocyte system where oleic acid totally inhibited haemolysis when control erythrocytes were 60% lysed.

Stabilizing effects of lipid-soluble compounds involve a hydrophobic interaction of the stabilizer with the membrane, and correlate best with the non-aqueous/aqueous partition coefficient than with any other parameter of the molecules. There is, however, invariably a point at which further increase in lipid solubi ity of a compound completely abolishes the stabilizing (as well as the anaesthetic) ability of the compound. This is known as the 'cut-off' effect[2].

Oleic acid methyl ester has a higher octanol/water partition coefficient than that of the acid or the alcohol[9]. If its lipid solubility classifies it among the compounds that have reached the 'cut-off' range than its inability to confer membrane stabilization might derive from the fact that very few molecules are found in the medium in a nonaggregated state. In such a situation, longer exposition time of the membrane to the compound might be needed in order to reach an effective concentration in the membrane.

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Figure 2 shows that whereas the acid and the alcohol at a concentration of 10^{-4} *M* caused an extensive rapid lysis of lysosomes suspended in 0.25 *M* sucrose, the ester exhibited its lytic effect only after an incubation for a period longer than 15 min. The remarkable lag period in the lytic effect of the ester could possibly reflect the need for its hydrolysis within the membrane domain by lysosomal lipases or non-specific esterases prior to exerting organelle damage. If this is the case, then high local concentration of free fatty acid will be the cause of lysosomal rupture. Under conditions where spontaneous lysis of lysosomes was recorded (37 °C, prolonged incubation, 0.25 *M*), it is plausible that membrane phospholipid hydrolysis which brings about the release of free fatty acids, may be the initiator for the damage to organelle intactness. Lysolecithin, the second product of phospholipid hydrolysis, has also been shown to have a biphasic effect on lysosomal integrity. The biphasic effect was observed when lysosomes were exposed to lysolecithin under the same conditions as applied for testing the effect of oleic acid (Figure 1) as well as when lysosomes were exposed to the phospholipid in 0.25 *M* sucrose for 2 h at 37 °C.

De Duve et al.[6] suggested that compounds causing lysis to lysosomes may act by initiating a sequence of events involving altered permeability to solutes, osmotic swelling and rupture. Induction of changes in membrane permeability may lead to the penetration of low molecular weight substrates rendering them available to lysosomal enzymes, while a high molecular weight solute may still be impermeable. Such differential permeability changes have been shown by Badenoch-Jones and Baum[13], who studied the effect of progesterone on the available and free acid phosphatase activity of lysosomes suspended either in sucrose or in polyethylene glycol (PEG, m.w. 1000).

We have tested the possibility of differential permeability changes in lysosomes exposed to 10^{-4} *M* of oleic acid. Figure 3 shows that within 15 min of exposure to oleic acid, 70% of total acid phosphatase activity was non-particulate while 100% of it was already available. Suspending the organelles in 0.25 *M* PEG (m.w. 1000, Carbide and Carbon Co., N.Y.) led to a reduction in both the available and free enzyme activity. At 15 min of exposure to the acid, about 42% of enzyme activity was non-particulate and 50% was available. Thus lysosomes, suspended in sucrose, reacted to oleic acid in the same pattern exhibited upon exposure to progesterone, but PEG did not seem to afford proection against rupture by oleic acid.

The observation that available activity in sucrose exceeds that in PEG (Figure 4) could derive from an expected difference in the initial properties of lysosomal suspensions in the two media. Alexandrowicz[14] presented evidence that high molecular weight polyethylene glycols, at high concentrations in solution have high osmotic coefficients; i.e. an increase from 1 to 5 was recorded for PEG of 6000 molecular weight. According to his theory, 0.25 *M* solution of PEG of a molecular weight of a 1000 would have a higher osmotic pressure than that of 0.25 *M* sucrose. The shift in the osmotic fragility curve, observed for lysosomal suspensions in PEG as compared to those in sucrose (Figure 4), is consistent with this interpretation. Thus in 0.25 *M* PEG the lysosomes are expected to shrink to a certain extent and therefore to be less prone to damage by oleic acid. The very small difference in the values of available and free acid phosphatase in PEG solutions implies that the primary interaction with oleic acid suffices to bring about leakage of enzymes and does not involve a change in permeability that allows substrate to permeate while PEG permeation is still retarded.

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Evidence of Mitotic Division of Coelomocytes in the Normal, Wounded and Grafted Earthworm *Eisenia foetida*

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Summary. In the oligochaete *Eisenia foetida* the free amoebocytes, once released into the coelomic fluid were observed to remain mitotically active following the trauma of receiving a body wall graft or wound.

There is general agreement[2–5] that the prime source of earthworm coelomocytes is the lining of the coelomic cavity. However, disagreement exists as to whether once free these coelomocytes are capable of dividing; several authorities[3, 6–8] have indicated that under stress there is an increase in the mitotic activity in the worm. However only one author[3] reported observations of mitotic figures in free coelomocytes, others[6, 7] reported this increase to be associated with the coelomocyte stem cells only. Several papers[7, 8] have indicated that cells in the cicatricial tissue which is regenerating and differentiating following trauma also exhibit mitotic activity. Contrary to these reports, Liebmann[2] observed no mitotic activity in the free coelomocytes and believed that they never undergo division. This latter view was recently supported by Cooper[9] who agreed that mitotic figures were never seen in coelomocytes.

In an attempt to resolve this problem, I examined coelomocytes for mitotic figures, in earthworms which had been subjected to wounding and a variety of graft combinations.

Autografts using *Eisenia foetida* var *unicolor*, allografts using *E. foetida* var *typica* and var *unicolor* and xenografts using *Lumbricus terrestris* as the donor and *E. foetida*

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