way. To 12.5 ml of 1:100 G.P. serum diluted in EDTA buffer at 37 °C were added 4.0 ml ice-cold EAC'1,4,2(Hu). The mixture was held at 37 °C for 2 min then 13.3 ml ice-cold EDTA buffer added and the suspension was centrifuged and drained. The cells were washed once with 13.3 ml ice-cold EDTA buffer. The cells were suspended in 4.5 ml of cold VBS, a zero time sample taken, and the suspension transferred to the 15 °C bath. The reaction was sampled as before. E* made from EAC'1,4,2(Hu) and Hu C'3 was prepared by adding 4.0 ml ice-cold EAC'1,4,2(Hu) to 13.5 ml 1:10 'M' at 37 °C. This mixture was held at 37 °C for 2 min, then the E* preparation was completed and the terminal transformation followed as described above. In addition, a control was included which lacked a C'3 source but was otherwise manipulated as described. The data are represented in Figure 2 and show again that E* prepared from G.P. C'3 completes the terminal reaction more rapidly than does E* made from Hu C'3.

Discussion. From Figures 1 and 2 it seems reasonable to suggest that the lower rate of the terminal reaction of Hu C' compared to G.P. C' is due to the EAC'1,4,2 + C'3 step. That is, the nature of the E* with regard to its lytic rate seems to be determined by the reactants which convert EAC'1,4,2 to E*. When EAC'1,4,2 cells are prepared using Hu C', the terminal reaction will assume the higher rate if G.P. serum furnishes the C'3. While EAC'1,4,2 made from G.P. C' will produce a slow-reacting E* if Hu serum is the source of C'3. The reaction EAC'1,4,2 + C'3 → E* has been shown to proceed by more than one step in both G.P.[10] and Hu C'[11] due to the existence of several C'3 subcomponents. Further study of the difference of the Hu and G.P. terminal reaction rates will require the use of C'3 subcomponents to define precisely the intermediate at which the difference emerges[12].

Zusammenfassung. Mit Meerschweinchen-Komplement sensibilisierte Schaferythrocyten (EAC'1,4,2) wurden einerseits mit Meerschweinchen-, andererseits mit menschlichem C'3 in Intermediärform (E*) gebracht. Sie wurden im ersten Fall rascher lysiert, als im zweiten Fall. Es wird vermutet, dass hauptsächlich die EAC'1,4,2 + C'3-Reaktion für die unterschiedliche Wirkung von Meerschweinchen- und menschlichem Komplement in der Terminalphase der Hämolyse verantwortlich ist.

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Phospholipid Catabolism in Some Organs of Rabbit During Aseptic Autolysis

Almost nothing is known at present about phospholipid catabolism. In some tissues, phospholipase activities were shown in vitro[1-6]: phospholipase A activity, which was found in different tissues, was made evident only against rat liver endogenous phospholipids[7,8]. But the data available do not furnish conclusive information about the pathways that operate in vivo; they deal with a latent activity, which is in balance and is probably masked in tissues, in vivo, by other enzymatic activities.

Using, as method of research, aseptic autolysis, we tried to show changes in phospholipid content of different organs, due to hydrolysis by phospholipase activity. During aseptic autolysis[9], many enzyme activities, cathepsins, and other acid hydrolases become free – activities which are not seen in intact cells. Therefore we thought that possible phospholipase activity might become manifest under these conditions.

Material and methods. Portions of rabbit heart, kidney and liver, removed from animals immediately after beheading and bleeding in sterile conditions, were kept in aseptic autolysis at pH 4.5, according to the method used by DIANZANI[9]. After 6 days of incubation at 37 °C, the tissues were removed, weighed, and extracted for lipid analysis. In order to exclude the possibility that phospholipids in the same experimental conditions were hydrolysed, aliquots of phospholipids extracted from normal tissues and standard phospholipid were incubated in the same conditions of tissues in autolysis with and without phospholipase A (as source of phospholipase A snake venom *Naja naja* was used, supplied by Miami Serpentarium, Florida), in final solution of 2.5 mg% in the same buffer acetate pH 4.5; the medium in which the phospholipid was incubated contained also bovine serum albumin (supplied by the Instituto Sieroterapico Toscano Sclavo) in final concentration of 0.065%. The samples incubated with snake venom were used as standard for lysolecithin, previous to extraction with chloroform-methanol (2:1).

Lipid extraction was carried out either on some portion of different organs not in autolysis used as control, or on tissue in autolysis. Tissue was extracted 3 times with 20 volumes of chloroform-methanol 2:1 (v/v) and the combined extracts washed according to FOLCH[10]. Phospholipids were separated by silicic acid impregnated paper

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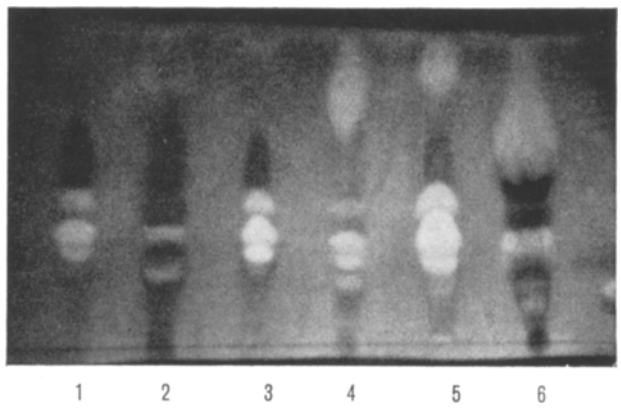
chromatography or by thin layer chromatography and identified, using a standard as reference. Lipids were stained with Rodamine 6G, and photographed under UV-light. Furthermore, lecithin and lysolecithin were identified with the test for choline[11]. Reagents used were products of Carlo Erba RS for chromatography; standards for lipids were supplied by Sigma.

Results. In the experiments reported it is shown that, during aseptic autolysis, phospholipids are destroyed in the same way (qualitatively) in heart and liver, while kidney seems to behave differently. The partial disappearance of lecithins and phosphatidilethanolamine and cardiolipin (diphosphatidilglyceride) and the contemporary appearance of lysolecithin and other lyso compounds can be seen: this appearance is much better demonstrated by the specific test not reported here for the amino groups and for choline. The controls, made by incubating phospholipid from normal tissue and phospholipid standard in the same condition of tissue in autolysis, show a partial destruction of cardiolipin and phosphatidilethanolamine (but not as clearly as in tissues in autolysis), and do not show destruction of lecithin, as we have, obviously, with lecithinase in the same way as during autolysis. From non-published data we found that there is a remarkable liberation of fatty acids and the quantity of these recovered is stoichiometrically superior to disappeared lecithin, and may partly come from hydrolysis of phosphatidilethanolamine and cardiolipin, or partly from hydrolysis of triglycerides of tissues. In the liver, the amounts of lysolecithin formed is equal to the lecithin that disappears, and the activity is higher than in kidney and heart.



Paper chromatograms according to MARINETTI to illustrate the differences in phospholipid composition of normal and autolytic rabbit tissues. The chromatogram was stained with Rodamine 6 G and photographed under UV-light. Spots 1 and 2 belong to heart phospholipid, 3 and 4 to kidney, and 5 and 6 to liver. Spots 1, 3, and 5 are phospholipid of normal tissue: the more polar component, the dark, spread-out spot at the top is cardiolipin; below cardiolipin the two light spots are from phosphatidilethanolamine and lecithin; the lower spots are in the region of sphingomyelin and phosphatidilinositol, but for these components there is much disagreement in the literature. Spots 2, 4, and 6, from the bottom upwards, are lysolecithin, lysophosphatidilethanolamine (in the area of phosphatidilinositol and sphingomyelin), lecithin, a non-identified spot, and cardiolipin. On the solvent front are migrated neutral lipid.

We can therefore say that during autolysis there is a phospholipase A activity in all three organs compared with the endogenous lecithin; we cannot yet say, however, that there is a hydrolytic activity against phosphatidilethanolamine and cardiolipin, because of their breakdown, as already mentioned, in the medium used for autolysis.

This is only a preliminary note, but we can anticipate that aseptic autolysis could be a good method for studying the pathways of endogenous lipid catabolism[12].

Riassunto. Gli autori hanno studiato il comportamento dei fosfolipidi durante l'autolisi aseptica di cuore, rene e fegato di coniglio. Dai risultati ottenuti concludono che si ha attività fosfolipasica in tutti e tre gli organi considerati verso le lecitine endogene del tessuto, attività difficilmente evidenziabile in vivo con altre tecniche.

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Excitation of Sham Rage Behaviour by Controlled Electrical Stimulation of Group I Muscle Afferents[1]

It is generally assumed that group I muscle afferents exclusively project onto segmental levels of the spinal cord and the cerebellum[2], with the possible exception of group I afferents from the forelimb which seem to project to the motor cortex as well[3]. In support of this opinion, high rate stimulation of group I fibres of the hamstring nerve could not induce electrocorticographic or behavioural arousal of intact unrestrained cats, nor could it discharge reticular units[4]. Since it is known that different effects may be obtained by working on preparations with different excitability backgrounds, we have tested muscle afferent stimulation in the acute decorticate animal.

Results. In 20 acute decorticate cats, 0.1 msec, 100–600 c/sec rectangular pulses were delivered to the central stump of a cut hamstring nerve through a bipolar collar-type electrode carrier, the proximal electrode being always the cathode. The stimulus threshold for the most excitable group I fibres was periodically checked by CRO monitoring from the sciatic trunk above the entrance of the hamstring nerve, and found to remain constant throughout the whole experiment. Stimulating voltages were always indicated as multiples of this threshold voltage (T meaning times threshold).