in the electrical response, ether evidently acts proximal to the contractile process of the muscle. The synergism shown between ether and curare might suggest that they act at the same site, but this does not necessarily follow. Since we do not yet know the exact point at which curare acts, further speculation as to the peripheral action of ether does not seem warranted.

As noted above, curare has been reported to cause greater "relaxation" of muscles during anesthesia with ether than during the same degree of anesthesia with other agents. While this suggests that there is a greater synergism of curare with ether than with these other agents, another possibility exists. Anesthetists judge the degree of anesthesia to a large extent by the respiration, and amounts of ether and other agents which depress the respiratory center to the same degree may have different effects at other centers, as well as on the neuromuscular junction. A detailed examination of the actions of various anesthetics would be necessary to settle this point.

Summary. The response of the gastrocnemius to maximal stimulation through its nerve was studied in the rat. During deep etherization there is a depression of 12% in the muscle action potential. At the same time the respiration is depressed; anoxia alone due to this may account for 5% depression. Hence a muscular depression of only 7% can be attributed to the action of ether, not necessarily involving neuromuscular block. Nevertheless there is a synergism between ether and curare, since the effects of these agents given together is one-third greater than the sum of their separate effects.

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Effect of Enzyme Inhibitors on Transformation of Enzymes in the Living Cell.

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Transformations in the enzymatic constitution of the living cell may occur as a result of gene mutation or as a physiological transformation in the presence of a constant genome. Changes of the latter type are most easily detected and studied in the cells of microorganisms, which are less specialized and more flexible than the cells of mature multicellular organisms, and present fewer complicating factors than the closely interrelated complex of cells found in the embryo. These changes have long been familiar to microbiologists; but they have until recently been the subject of much controversy, centering around the question whether they are only apparent transformations resulting from the action of natural selection upon pre-existing mutants in microbiological populations. Recent developments in the genetics of yeast[1] have made it possible to settle the controversy at least for this group of organisms; and it has been demonstrated by Spiegelman and coworkers[2,3] that modifications of enzyme constitution take place in genetically stable yeast strains, without the intervention of natural selection and without cell multiplication.

Most domestic strains of yeast readily ferment glucose, but do not ferment galactose. Some of them can, however, acquire the ability to ferment galactose after

[1] Lindegren, C. C., *Bact. Rev.*, 1945, **9**, 111.

[2] Spiegelman, S., Lindegren, C. C., and Hedgecock, L., *Proc. Nat. Acad. Sci.*, 1944, **30**, 13.

[3] Spiegelman, S., and Lindegren, C. C., *Ann. Mo. Bot. Gard.*, 1944, **31**, 219.

TABLE I.
Effect of NaN_3 on Galactose Fermentation.

Time	Azide conc.:	Control	10^{-5} M	10^{-4} M	10^{-3} M
		Unadapted cells.			
90-180′	Microlitres	138	242	28	26
	% of control	—	175	20	19
		Adapted cells.			
10-120′	Microlitres	944			1314
	% of control	—			139

a period of exposure to this sugar under aerobic conditions. The time required for "adaptation" (a term introduced by Karström[4] to denote this process) is a characteristic constant for each strain of yeast. It is this enzyme transformation which has been most extensively studied by Spiegelman, and which is the subject of the present report.

The effect of several well-known enzyme inhibitors on the adaptation to galactose fermentation has been studied in the hope of casting further light on the nature of the process. In particular, we wished to obtain evidence bearing on the following crucial question: Is adaptation the synthesis of one or several new enzymes or coenzymes by the cell, or is it merely a lag within the metabolic cycle of the substrate itself, involving the formation of a certain level of intermediate products (*e.g.*, phosphorylated intermediates), as in the well-known induction period which occurs when glucose is fermented by dried yeast? Our results point strongly to the former alternative—the occurrence of synthesis.

Materials and Methods. A pure strain of *Saccharomyces carlsbergensis* (CLD-1A, obtained from Dr. S. Spiegelman) was grown in liquid culture containing peptone and 8% glucose, together with yeast extract and the necessary salts. For experiments, 48-hour cultures were harvested, washed several times with M/15 KH_2PO_4 to remove adherent medium, and finally suspended in enough M/15 KH_2PO_4 to give about 20 mg (wet weight) of yeast in 1 ml of suspension. Aliquots of 1 ml were distributed among conventional Warburg reaction vessels; enough galactose solution to make a final concentration of 4% was placed in the side-arm of each vessel; and enough distilled water, inhibitor or phosphate solution was added to the main chamber to bring the final volume of liquid to 2 ml. In occasional experiments the concentration of yeast or of galactose was varied, but in any one experiment the conditions in all vessels were strictly comparable. After equilibration on the Warburg bath, the substrate was tipped into the main chamber, the time at which this occurred being designated as zero time. Oxygen consumption and CO_2 production were followed at 15-minute or sometimes at 10-minute intervals by the usual 2-cup method.[5] The excess of CO_2 production over O_2 consumption in a given time period was taken as an index of fermentation and hence of adaptation rate. Inhibitor solutions were adjusted to a pH of 4.5 before use.

Experimental Results. The inhibitors chiefly used were sodium azide (NaN_3), sodium fluoride (NaF), 2-4-dinitrophenol (abbreviated DNP), and iodoacetic acid (abbreviated IAA). Their effects on unadapted and fully adapted cells were examined. Adapted cells were obtained by growing the yeast in the usual liquid medium with galactose substituted for glucose.

Azide is known as an inhibitor of hemin catalyses,[6] but also (unlike HCN and H_2S) as a specific inhibitor of syntheses even under anaerobic conditions.[7,8] This makes it particularly useful for our purpose.

The adaptation time of our yeast strain

[4] Karström, H., *Erg. Enzymforsch*, 1938, **7**, 350.

[5] Dixon, M., *Manometric Methods*, University Press, Cambridge, 1943.

[6] Keilin, D., *Proc. Roy. Soc. Lond.*, B, 1936, **121**, 165.

[7] Spiegelman, S., *Biol. Bull.*, 1945, **89**, 122.

[8] Winzler, R. J., *Science*, 1944, **99**, 327.

being 90 minutes, data obtained from unadapted cells are given only from 90 minutes on. With fully adapted cells, only the highest inhibitor concentration was used. Table I shows a typical set of results.

Despite the fact that the O_2 consumption (not shown) was just as completely inhibited by 10^{-3} M azide in adapted as in unadapted cells, the fermentation of galactose by adapted cells was not only not inhibited, but was actually stimulated by azide. None of the other compounds used gave such results; and we therefore present the data for them somewhat more briefly. Concentration curves were obtained, but we show here only the data obtained at the highest concentrations used. The stimulatory effect of azide at low concentrations will be discussed more fully elsewhere.

As in the case of azide, the effect of these compounds on O_2 consumption was very nearly the same for adapted and unadapted cells. Unlike azide, however, they inhibited the galactose fermentation equally well in both cases, with the exception of DNP. The results therefore leave open the question of the mechanism of inhibition.

TABLE II.
Effect of NaF, IAA, and DNP on Galactose Fermentation.
(Excess CO_2, % of control).

NaF 2×10^{-2} M	IAA 2×10^{-4} M	DNP 10^{-3} M
	Unadapted cells.	
0	14	3
	Adapted cells.	
6	0	40

We therefore attempted to determine whether the inhibition of unadapted cells could be reversed. Cells were exposed to the inhibitors for 90 minutes in the presence of galactose, then carefully washed free of adhering solutions, and tested for fermentation on fresh galactose. If any adaptation had occurred, the washed cells should have begun to ferment before 90 minutes. The results from 60 minutes onward are given.

With the possible exception of fluoride, the results appear negative. In the case of IAA, however, irreversible changes tend to occur after long periods of exposure. Fluoride[9] is known to combine with magnesium and phosphate as a complex salt within the cell, this complex then uniting with the enzyme enolase; and it is not certain that this complex would have been removed by washing the cells. Consequently, the results of Table III must be regarded with some reservations.

TABLE III.
Reversibility of Inhibition of Adaptation.
(Excess CO_2, % of control).

NaF 2×10^{-2} M	IAA 2×10^{-4} M	DNP 10^{-3} M
17	2	3

Discussion. The most clearcut results with inhibitors have been obtained with sodium azide. This poison prevents adaptation from occurring, but does not prevent galactose fermentation after adaptation has been allowed to take place. It does not interfere with the metabolic chain by which galactose is transformed into alcohol and CO_2. It must therefore prevent the formation of one or more essential enzymatic or coenzymatic links in that chain. This supposition is reinforced by the knowledge that azide does in fact inhibit synthetic processes, and does not inhibit normal glucose fermentation.

The poison DNP inhibits adaptation completely, but inhibits galactose fermentation by adapted cells to the extent of only 60%. It is known to have an effect on synthesis[10] even at rather low concentrations; and it may in these experiments be functioning in a mixed way—to inhibit synthesis of the adaptive enzyme or enzymes, and to block or slow up part of the process of fermentation.

The compounds NaF and IAA are known to block the Meyerhof fermentation scheme.[9,11] It is therefore likely that they merely prevent the adaptive enzymes from manifesting themselves, without necessarily preventing the adaptive enzymes from being formed.

[9] Warburg, O., and Christian, W., *Bioch. Z.*, 1942, **310**, 384.

[10] Clowes, G. H. A., and Krahl, M. E., *J. Gen. Physiol.*, 1943, **21**, 77.

[11] Lundsgaard, E., *Bioch. Z.*, 1930, **217**, 162.

However, the data on this point are as yet not conclusive.

From the effects of azide in particular we conclude that adaptation to galactose fermentation involves the synthesis of one or more compounds which are not metabolic intermediates of galactose utilization, probably enzymes or coenzymes.

Summary. The effects of several enzyme inhibitors on the adaptation of yeast to galactose fermentation has been studied. The results strongly indicate that the process of adaptation involves the synthesis of one or more new enzymes.

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Influence of an Alkaline Solution on Tissue Toxicity of Uranium Nitrate.

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The observation was made in this laboratory[1] that a solution of sodium carbonate would not only protect the kidney against the toxic action of a uranium salt but that it would furthermore protect it against the injurious effect of a general anesthetic body. The first part of this observation was confirmed by Goto[2] and very recently further extended by the studies of Donnelly and Holman[3] who were able to demonstrate a similar order of protection from a solution of sodium citrate. In a later paper[4] these authors and their associates attempt an explanation for this phenomenon of protection. They attribute it not to the action of a solution of sodium citrate as an alkaline medium but they believe that their "data on sodium citrate protection against uranium injury point to the maintenance of one or more vital equilibria (possibly the 'citric acid cycle' of carbohydrate metabolism) while the normal processes of repair are taking place, for we have no evidence that repair is accelerated or altered in any way." It becomes difficult to see how these authors could come to such a conclusion without employing control experiments in which sodium carbonate[1] or sodium bicarbonate[2] were used as agents to induce protection against uranium. Such an observation is especially appropriate when the fact is well established that citrates are rapidly and completely changed in the tissues to carbonates.

The way in which an alkaline solution protects the kidney, the liver and likely other tissues against a uranium injury is not known. It is furthermore not known how uranium induces its injury. Holman and Douglas[5] have shown they were able to recover from 31 to 88% of uranium nitrate from the urine of dogs during the first 24 hours of an intoxication by 5 mg of this substance per kg of weight. It is at this period that the renal injury commences and rapidly progresses. The suggestion is here repeated[1] that the cellular toxicity of uranium salts may be due to their ability to inhibit processes of intracellular oxidation and that such an inhibition is due to the characteristic of the uranium atom to be radioactive. Such an inhibition would lead to an intracellular accumulation of hydrogen ions with a change in the chemical environment in which various oxidation reduction systems exert their

1 MacNider, Wm. deB., *J. Exp. Med.*, 1916, **23**, 171.

2 Goto, Kingo, *J. Exp. Med.*, 1917, **25**, 693.

3 Donnelly, G. L., and Holman, Russell, *J. Pharm. and Exp. Therap.*, 1942, **75**, 11.

4 Donnelly, G. L., Ross, C. J., Meroney, W. H., and Holman, Russell L., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 75.

5 Holman, Russell L., and Douglas, William A., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 72.