possible that this increased turnover is associated with the increased oxidation of glucose that insulin produces in resting muscle. Such coupling of phosphorylation with oxidative reactions has been reported many times in cell-free extracts. In most of such studies the emphasis has been placed on adenosine triphosphate, but the present findings indicate that phosphocreatine is also involved. Obviously it is inadvisable to attempt any specific correlations between the observed changes in the intact animal and the vast number of reactions found in cell-free extracts. However, these results point strongly in the direction of such an association of phosphorylation with glucose oxidation in the resting metabolism of muscle, and indicate that insulin, by accelerating glucose oxidation, also accelerates these phosphorylation reactions.

With regard to glucose-6-phosphate, on the other hand, it is evident that the administration of glucose alone to the 24-hour fasted animal does produce an increased turnover which is not affected by an external supply of insulin. It remains for future study to determine whether the increased turnover evoked by glucose depends on the normal secretion of insulin.

Caution is necessary in evaluating the data on the glucose-6-phosphate in relation to the question of phosphorylation during glucose absorption. The presence of P^{32} in higher concentration in this substance than in the other organic phosphorus compounds shows that some phosphorylation of glucose does take place. However, in relation to the total probable glucose absorption, the amount of phosphorylation observed is rather small. This becomes evident on comparison of the P^{32} levels of glucose-6-phosphate and plasma inorganic phosphate. The average glucose-6-phosphate content of resting muscle does not exceed 10 mgm per cent., calculated as P, and there is no interchange of phosphate groups between this substance and phosphocreatine or adenosine triphosphate.[1] Therefore the transfer of 1 mgm per cent. of P across the cell membrane in the form of glucose-6-phosphate should raise the P^{32} level of this substance to at least 1,000 counts per minute per mgm P when the P^{32} of the plasma P is of the order of 10,000 counts per minute per mgm P. But the highest values obtained are only about half of this amount, indicating the transfer of about 3 mgm per cent. of glucose into the muscle in the form of glucose-6-phosphate. This holds even in the insulin experiments, when it would be anticipated that glycogen deposition and glucose oxidation are taking place at relatively high rates. If the glucose phosphate were dephosphorylated in the formation of glycogen, not only the glucose-6-phosphate but also the inorganic phosphate of the muscle should show higher P^{32} levels in the insulin experiments than in the other two groups. Two possible interpretations of the present data are: (1) that the absorption of glucose by the muscle fiber does not involve the entrance of a phosphate group into the cell, or (2) that glucose-6-phosphate is not involved in the principal mechanism of glucose absorption by resting muscle.

Summary. Insulin causes a marked increase in the turnover rates of phosphocreatine and adenosine triphosphate in resting muscle during glucose absorption, as determined with radioactive phosphorus, but does not cause any increase in turnover of glucose-6-phosphate beyond that produced by glucose itself.

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JACOB SACKS

DEPARTMENT OF PHARMACOLOGY,
UNIVERSITY OF MICHIGAN MEDICAL SCHOOL

SULFONAMIDE DEPRESSION OF INORGANIC CATALYTIC ACTION[1]

AN anti-catalytic action has been urged as the fundamental mechanism of the antiseptic action of the sulfonamides, although it would probably be weaker than that of many other antiseptics. The evidence for this has been limited to catalysts in living microorganisms. This theory does not necessarily conflict with the competition-interference theory in which the utilization of para-aminobenzoic acid and other agents essential to bacterial growth is affected by sulfonamides, because metabolic functions are basically mediated by catalytic action. Depression of all catalysts by the sulfonamides would be expected to decrease metabolism and growth of microorganisms. Johnson[2] has reported that the activity of the catalyst luciferase is reduced by sulfonamides and a number of other chemically unrelated agents, which, however, possess in common a depressant pharmacological action, especially narcosis. Certain narcotics, alkaloids, antiseptics and toxic ions are known for their depressant actions on inorganic catalysts, which are wholly foreign to living microorganisms. Thus far, however, no test has been made for similar possible effects of the sulfonamides. If positive, this would help to establish a general anticatalytic action for these chemotherapeutic agents. This report presents positive results, demonstrated by us with three inorganic catalysts, namely, fullers' earth, platinum black and colloidal silver.

Methods: Santesson's[3] procedure for testing cata-

[1] From the Department of Pharmacology and Therapeutics, Stanford University School of Medicine, San Francisco, Calif.

[2] Johnson, SCIENCE, 95: 104, 1942.

[3] Santesson, *Skand. Arch. f. Physiol.*, 42: 129, 1922.

lytic liberation of oxygen from hydrogen peroxide *in vitro* was used. Quinine and a number of other protoplasmic poisons known to inhibit this catalytic action were used for checking the validity of results with the sulfonamides and other compounds. In each experiment there were results on oxygen liberation with the unpoisoned catalyst, as control, for comparison with results with a number of agents under the same conditions. The procedure was as follows: Into a small fermentation tube a weighed and constant amount (20 mgm or less) of the catalyst was placed. Next, a small, though constant, amount of the agent to be tested was added, except in the controls (usually 1 cc of a 1 per cent. solution, or less). Then, 1 cc of a 1 per cent. solution of hydrogen peroxide was added. Finally, the tube was filled with water, and quickly inverted into a slightly larger tube, also filled with water. As the oxygen was liberated it collected in the smaller tube and the height of the column of gas was measured by comparison with a millimeter scale.

Three different catalysts were used, namely fullers' earth, which caused a slow liberation of oxygen; platinum black, which caused a rapid, almost explosive, liberation; and colloidal silver (collargol), which had intermediate activity. All gave similar results, but fullers' earth was used in the vast majority of experiments, as the slow action prevented loss of liberated oxygen during manipulation of the tubes. The action was somewhat capricious and necessitated use of accurate quantities throughout for satisfactory results. For each agent tested, multiple trials were made, usually 10 or more. There was a general tendency for the gas formation in both test and control tubes to reach eventually the same volume, and, therefore, the tubes were read at various times before the total completion of catalytic action. When platinum black and collargol were used, the gas-measurements were made at the end of 5 minutes, or less; with fullers' earth, at the end of 24 hours.

Comparative Effects of Various Protoplasmic Poisons and Sulfonamides: Quinine sulfate, quinacrine hydrochloride, pamaquine naphthoate (suspension), acriflavine, sodium salicylate, carbarsone and sodium cyanide markedly reduced the liberation of oxygen, generally less than 50 per cent. of the control volume. Free acids (sulfuric, ascorbic, nicotinic and para-aminobenzoic) were also strongly inhibitory, and therefore all agents with an acid reaction were carefully neutralized before trial. Alkalis (hydroxide and bicarbonate of sodium) had no effect, or increased slightly the liberation of oxygen. Accordingly, the effects of sodium salicylate and sodium cyanide, which are alkaline, must have been due to the salicyl and cyanogen ions, respectively. The single exception to the general group of protoplasmic poisons was sodium fluoride, which increased markedly the liberation of oxygen with fullers' earth and collargol. However, fluoride reduced the activity of platinum black, about like quinine. Platinum black and collargol were affected like fullers' earth by all other agents tried.

Sulfanilamide and sulfaguanidine were less active as depressants than the most potent poisons tried as they reduced the volume of liberated oxygen only about 16 per cent. The other sulfonamides tried, sulfapyridine, sulfathiazole, sulfadiazine, sulfacetimide and sulamyd, were more active, the liberation of oxygen being reduced from 22 to 54 per cent., despite their lower solubility than sulfanilamide. The sodium salts of acetylsulfanilamide and of acetylsulfapyridine were less effective, causing about 20 per cent. reduction in oxygen liberation. The sodium salts of sulfanilamide, sulfapyridine, sulfathiazole and sulfadiazine generally produced weaker effects than the free compounds, *i.e.*, about 20 per cent. less. The depressant effects of the sulfonamides occurred on all the three inorganic catalysts and were consistent in individual group tests, but varied from group to group, the per cent. changes reported being medians. It appeared interesting to determine next the effects of so-called "inhibitors" of sulfonamide action on the anticatalytic effects of these and some other drugs.

Action of "Inhibitors" on Anticatalysts: This was determined with fullers' earth only. Neutralized solutions of para-aminobenzoic acid alone had practically no demonstrable effect on oxygen liberation. When sulfonamides were also present, or quinine, pamaquine or quinacrine, there was no interference with their anticatalytic actions. Neutralized nicotinic acid had a strong depressant action on oxygen liberation, which was practically not affected by the presence of sulfonamides or the quinine group.

Comment: These results place the sulfonamides in the group of anticatalysts affecting non-living catalytic systems. This agrees with the claims of others as to an anticatalytic action in microorganisms being fundamental to the antiseptic action of these agents. The mechanism of this anticatalytic action is uncertain, but may be a physico-chemical surface phenomenon. Compared with the quinine group of alkaloids and a number of other protoplasmic agents, the sulfonamides are weak depressants of catalytic action, which is consistent with their known action as systemic antiseptics. Para-aminobenzoic acid, nicotinic acid and the sulfonamides are non-competitive for the inorganic catalysts used, as the nutrient agents do not demonstrably inhibit the sulfonamide depression. Nor does the latter involve replacement of the nutrient agents, since these are absent in the inorganic catalyst, unless purposely added. This lack of antag-

onism of the para-aminobenzoic acid and nicotinic acid on sulfonamides in inorganic catalysts is a difference from the conception of the antagonism or competition in living systems. However, this may be more apparent than real, as there are well-known difficulties in reconciling the actions of these agents and their properties and the relationships of antagonisms which need not be considered here. Certainly, it is of more than passing interest that the sulfonamides can reduce inorganic catalytic actions like other antiseptics and poisons.

CONCLUSIONS

(1) The sulfonamides decrease the liberation of oxygen from hydrogen peroxide by such inorganic catalysts as fullers' earth, platinum black and collargol, but the depression is much weaker than that caused by quinine, pamaquine, quinacrine and a number of other protoplasmic poisons. This agrees with current theories of an anticatalytic action as being fundamental to the antiseptic action of these agents.

(2) Para-aminobenzoic acid and nicotinic acid and the sulfonamides are non-competitive, or not antagonistic to each other, on the inorganic catalysts used, a difference from their action on microorganisms.

P. J. HANZLIK
W. C. CUTTING

GROWTH STIMULATION BY AMMONIUM SULFAMATE IN LOW CONCENTRATION

IN laboratory experiments on the toxicity of ammonium sulfamate $NH_4O_3SNH_2$ for nut grass (*Cyperus rotundus* L.),[1] it was observed that the poisonous action of the salt was preceded by a marked increase in the number of plants during the first three days of the experiment. Indications of a growth-stimulating action have been discussed also in investigations of other herbicides, *e.g.*, the chlorate,[2] the sodium bromate[3] and the thiocyanate.[4] The latter one has already found application for hastening the germination of seeds and potatoes.

It seemed worth while to investigate the possible stimulating effect of the ammonium sulfamate. Duckweed (*Lemna minor*) was selected as a test plant as in former experiments[3] because the increase in the number of plants offered a convenient way of watching the effect of the compound. The plants (on the average 20) were kept in glass jars filled with two inches of soil and two liters of tap water. The number of plants was counted daily and expressed in per cent. of the initial number. The curves reproduced below give the average of all the experiments performed during several months.

Curves 1 and 2 represent the effects of so much ammonium sulfamate as to make the solution 0.01 and 0.005 molar with respect to this salt (Fig. 1). In both

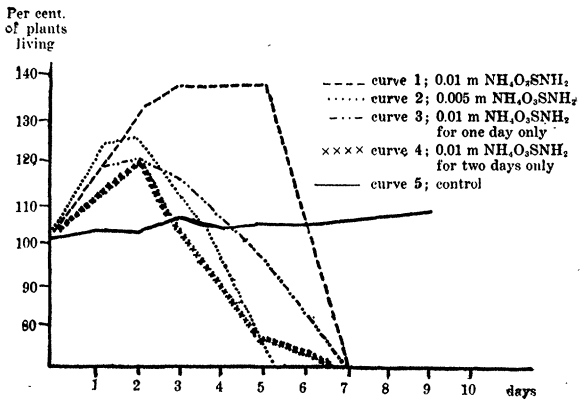

FIG. 1

cases the increase in number over the control experiments (curve 5) is very remarkable for the first 2 to 5 days, but then the poisonous action of the salt leads to a rapid and complete destruction of all the plants.

It seemed possible that a reduction of the time of action of the salt on plants might lead to a separation of the stimulating and the toxic effect. In further experiments the plants were therefore removed from the sulfamate-containing jar after a limited period of time and transferred to another one containing tap water and soil only. Curves 3 and 4 show that an action of the sulfamate for two- or one-day, respectively, reduced mainly the stimulating effect but did not alter appreciably the rate of poisoning. However, the killing of the plants could be avoided when the contact with the sulfamate was limited to five hours (curve 6, Fig. 2). The increase in the number of

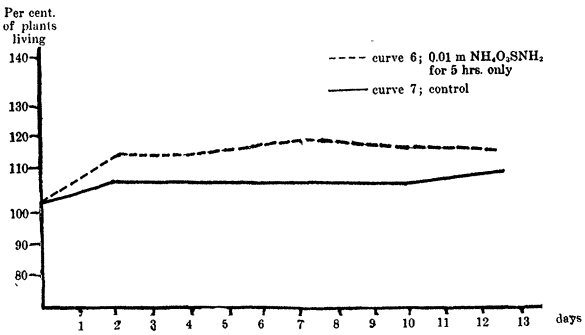

FIG. 2

plants was small under these circumstances, in fact only about one fourth of that obtained in the first experiment, but the rate of growth in the first two

[1] Fromm, *Ciencia y Técnica*, 1: 69, 1943.
[2] Shear, *Phytopathology*, 25: 440, 1935.
[3] Hessenland, Fromm and Saalmann, *Angew. Chem.*, 46: 577, 1933.
[4] Denny, *Biol. Abstracts*, No. 674, 1930.