THE ANODIC OXIDATION OF BENZENE.

B.

THE EFFECT OF CERTAIN CHEMICALS ON THE HYDROLYTIC ACTIVITY OF RICINUS AND PORK PANCREAS LIPASE.

BY

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A.

THE

ANODIC OXIDATION OF BENZENE.
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I. Introduction.

Purpose:

The investigation was carried on with a view to finding (1) whether or not there is truth in the predictions of several organic electrochemists, that on electrolytic oxidation of benzene the product first formed is phenol, and if this be true, then to discover (2) what factors influence the yield of this product, thereby determining by what means the yield can be increased.

Summation of factors to be considered:

Of the two reactions encountered in organic electrochemistry, viz. oxidation and reduction, the former is the least developed. The greater difficulty experienced in controlling the factors which influence the reactions of organic electro-oxidation have led, in the past, to intricate courses of reactions, resulting in low yields and disappointment. Hence electro-organic chemistry has not been regarded as a very fruitful field for commercial manufacture. Especially is this true in the case of aromatic compounds. For, the fatty compounds, being, as a rule, the more readily oxidized, break up in stages till 1. Trans. Am. Electrochem. Soc., 40, 109, (1921).
finally, they are converted into carbon dioxide and water, whereas the aromatic compounds form phenolic condensation products of a resinous nature. Though difficulties have been many, and the results, as a rule, disappointing, nevertheless, experiments in this field have been numerous and some, especially those involving the use of catalysts, have led to interesting results. It has long been known that anodic oxygen oxidizes aromatic compounds by the introduction of hydroxyl groups. If the reaction at the anode could be sufficiently controlled, benzene could be converted into the relatively more valuable commercial product, phenol.

Fichter and other electro-organic chemists believe that phenol is the first oxidation product of benzene. Brockman ¹ refers to a work of Fichter and Uhl ² in which these last, using benzene with a twice normal solution of sulphuric acid at 60° in an atmosphere of carbon dioxide, obtained at a platinum anode, a small quantity of phenol. In a later work Fichter ³ states that he was unable to prove that phenol is the first oxidation product of benzene. A search of the available literature has brought to light no other cases where phenol has been obtained electrolytically

from benzene itself.

However, Hoppe-Seyler \(^1\) obtained from benzene, water, and palladium hydride sponge in the presence of air, sufficient phenol to detect qualitatively. Leeds \(^2\) observed that in time, a very dilute solution of hydrogen peroxide converted benzene directly to phenol. Nencki \(^3\) found that oxygen of the air in the presence of sodium hydroxide converted benzene to phenol. Cross, Bevan and Heiberg, \(^4\) by using ferrous sulphate with two molecular weights of hydrogen peroxide to one of benzene, oxidized 10 g. of benzene obtaining 1.5 g. of phenol. Undoubtedly this indicates that phenol is the first, (or at least one of the first), products of oxidation of benzene. The fact that oxidation with hydrogen peroxide yields, very often the same products as does anodic oxygen, together with the fact that hydrogen peroxide oxidizes benzene to phenol, would lead one to expect to find phenol as one of the products of electro-oxidation of benzene.

There are several reasons for the fact that under ordinary conditions phenol has not been detected as a product of electrolysis. First, the process of electrolytic

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1. Ber. 12, 1552.
2. Ber. 14, 975.
4.

oxidation is so hard to control that the reaction proceeds past the phenol stage, and higher oxidation products are formed. Second, benzene is highly resistant to oxidation being oxidized appreciably only by ozone, hydrogen peroxide, mono persulphuric acid, and by the most effective of all oxidizing agents, anodic oxygen. Phenol, on the other hand is relatively more easy to oxidize. The more the benzene nucleus is saturated with oxygen, the more readily decomposition into oxalic acid, carbon dioxide, etc., takes place. Hence phenol, as soon as it is formed, being a better depolarizer than benzene, is almost completely oxidized to higher products before more benzene is converted into phenol. Or, in other words, with respect to certain compounds, there is an equilibrium set up in which the concentration of phenol is very low. Third, benzene in most electrolytes, is much less soluble that phenol. Hence a sufficient concentration of reactant cannot be obtained to act as a good depolarizer. As a result, the anode reaction is slow and incomplete. The product phenol, however, being quite soluble in the electrolyte, takes the place of the benzene as a depolarizer and is oxidized to a higher state. Fourth, as a result of the insolubility of benzene, it is impossible to regulate closely the voltage to the oxidation potential, i.e., to the voltage just sufficient to oxidize benzene to its first stage of oxidation. Failure to overcome these difficulties has led experimenters to obtain
5.

from benzene the following products.

\[
\begin{align*}
\text{p-p'} & \text{ diphenol} \\
\downarrow & \\
\text{quinone} & \leftarrow \text{hydroquinone} \\
\downarrow & \\
\text{with diaphragm} & \\
\text{maleic acid and p-benzoquinone} & \leftarrow \text{formaldehyde succinic} \\
\text{racemic acid and formic and} & \text{oxalic} \\
\text{tartaric acids and} & \text{or with diaphragm} \\
\text{carbon dioxide and} & \text{fumaric} \\
\text{C}_4\text{H}_4\text{O}_5 & \text{and C}_7\text{H}_6\text{O}_4 \text{ m.p. 93} \\
\text{Also n-valeric and malonic acids, o-hydroxyphenol, ethers,} \\
\text{diphenyltetrahydroxydiphenol, dihydroxydiphenol ester,} \\
\text{phenolic resins, etc.}
\end{align*}
\]

There appear to be two possible ways of stopping the oxidation as soon as it reaches the phenol stage. First by removing the phenol from the electrolyte as soon as it is formed and second, by moderating the intensity of the electrolytic oxidation. So far, the first step has not been accomplished. Distillation of phenol even from a solution of a stronger acid does not take place (unless the latter has a higher boiling point), since the concentration of the phenol is so very small. Precipitation of the phenol, or some compound of phenol from the electrolyte would shift the reaction equilibrium in the desired direction and would prevent further oxidation, since the phenol could no longer act as a depolarizer. But all known means of precipitation are either too expensive, or else interfere
with the desired reaction or electrolysis. So far, no means of washing out the phenol from the electrolyte by the use of another liquid immiscable with the anolyte, which is suitable to this particular process have been devised.

The second expedient, viz. that of moderating the intensity of electrolytic oxidation seems most useful. There are many ways of doing this, and the most of these depend on the regulation of the voltage used,--hence also of the effective pressure and concentration of anodic oxygen.

The first and most obvious way is to increase the concentration of the benzene in the electrolyte so that benzene rather than phenol will act as the depolarizer. In the past, the electrolysis of benzene has been carried on in dilute sulphuric acid solutions in which benzene is insoluble. It is probable that the primary products are the same with alkaline solutions as with sulphuric acid solutions except that new complications set in owing to the instability of quinone in alkaline solutions.

Unfortunately most electrically conductive solvents and aqueous electrolytes do not dissolve benzene, and according to Fichter, even the fine emulsions produced by vigourous stirring are far from being as effective as true solutions. However Brockman\(^1\) says that a soluble catalyst is sufficient to make up for a poor solvent. Sodium acetate\(^2\) and

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alcohol have been used to increase the solubility of the reactant, but the first substance does so only slightly and the latter becomes itself, the depolarizer. The problem then, is to find another more suitable solvent. It is to be expected that the product will vary with the solvent used. The ideal solvent for this reaction would

(1) dissolve large quantities of benzene,
(2) have a low specific resistance,
(3) be not too volatile,
(4) be one in which phenol is insoluble, (since its removal from the solution prevents further oxidation).
(5) be attacked only slightly by the electric current.
(6) be miscible in all proportions with water, and
(7) if possible, be inexpensive.

Pyridine, especially with aromatic compounds, has a tendency to form tarry substances which result in loss of material. Glacial acetic acid to which has been added a small amount of water fulfills all the above requirements except (2) and (4). This will not conduct the electric current appreciably, (not nearly as well as will a liter of water with a few cc. of acetic acid). But if instead of water, a few cc. of concentrated sodium hydroxide solution are added, the solution becomes a good conductor of electricity and the benzene is still soluble. The acetic acid is

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only slightly attacked. Bourgoin found that of all the acids tried, acetic was the hardest to electrolyze, especially when concentrated. The glacial acid diluted with an equal volume of water gave 29.7% of oxygen and 2.3% of carbon dioxide. Hopfgartner using anhydrous sodium acetate in glacial acetic acid with platinum electrodes got 49.28% carbon dioxide, 1.17% ethylene, .16% of oxygen, 21.06% of ethane and 27.85% of hydrogen (by volume). Warming and the presence of alkali decreased the yield of ethane. Other products are carbon dioxide, methyl acetate, methyl carbonate, methyl formate and formic acid.

The second means of moderating the intensity of oxidation is by decreasing the overvoltage of oxygen at the anode and the oxidation potential of the electrodes. The oxidizing power of an anode depends, in part, on the potential difference between the anode and the solution. The decrease in oxygen overvoltage can be accomplished, to some extent, by the superimposition of alternating on direct current. Goodwin and Knobel have studied the effect of alternating current on overvoltage, at platinum, copper, and lead electrodes. By the use of a variable frequency generator which gave a sinusoidal current wave, they experimented over the range of 2 to 100 cycles per sec.

They found that the decrease in overvoltage was a function of the ratio of alternating current and was independent of the material of the electrodes and of the current density. They state that the effect is negligible when the ratio is less than 0.7 and reaches a maximum when the ratio is 3.2. There is, however, some difference of opinion as to whether or not there is a minimum value which must be exceeded before an appreciable effect is produced. The effect increased slightly, (50mv. over the range studied), as the frequency was diminished. The power factor has a small influence, the change from 0 lead to 0.5 lag giving a difference of 30 mv. The decrease of irreversibility of the direct current electrode process is said by Cooper to produce remarkable effects at frequencies below 10 cycles per sec. By the superimposition of alternating and direct current, the retardation phenomenon and passivity in anodic metal solution can be eliminated and the limiting current density at which metal solution ceases can be raised. Though most of the work has been concerned with the effect of overvoltage of hydrogen at the cathode, Grube and Dulk observed that a high ratio of alternating current lowered considerably the overvoltage of oxygen at platinum anodes. Oxygen was obtained at a potential less positive

than the reversible value. Marie \(^1\) found that when oxidizing alcohol the voltage at a platinum anode in an acid medium rose from .7 to 1.1. He could carry on the oxidation at the lower voltage by using an intermittent current. This simply prevented polarization. Reitlinger, \(^2\) by using alternating current superimposed on direct current, was able to reduce the polarization to such an extent that an alcohol gave the corresponding aldehyde rather than the acid which resulted when direct current only was used. That is, by the use of alternating on direct current, the effective voltage or oxidation potential is lowered so that products can be obtained which are intermediate between the reactants and the products obtained with direct current alone. These phenomena all point to the possibility of regulating the intensity of oxidation by the superimposition of alternating on direct current, \(^3\) but with platinum anodes this expedient had the disadvantage of causing excessive corrosion of the electrodes.

An expedient closely associated with this is the use of an intermittent, (though not alternating), current. In the following experiments such a current has been obtained by the use of tantalum electrodes in acid solution. The alternating current of the desired voltage was applied.

\(^1\) Chel Obst., 32, 4345. Compt Rend.
The tantalum electrode almost completely rectified this current.

The third means of moderating the intensity of the electrolytic oxidation, related in some ways to the second, is in the choice of proper anode material. The choice of conductors for an anode is rather limited,---much more limited than in the case of the cathode,---since many metals suitable for cathodes are attacked at the anode. The choice for alkaline solutions is limited to platinum, palladium, iridium carbon, iron, and nickel, (in the absence of ammonium salts). In acid solutions we can use carbon in the form of Acheson graphite (in solutions containing Cl⁻), platinum (in absence of halides), and lead or lead peroxide (in sulphuric acid solutions with specific gravity up to 1.6, or solutions with any concentrations of phosphoric acid, neutral sulphates, phosphates, carbonates and chromates). Carbon has the disadvantage of disintegrating mechanically and magnetite has too low a conductivity. Copper is a suitable anode for moderate oxidations, but is slowly attacked and deposited on the cathode. Oxidation is said to proceed most violently at smooth platinum anodes. Next to these are lead dioxide anodes. Iron and nickel (especially spongy nickel) anodes have the lowest overvoltage as shown by the

The following table.

<table>
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<tr>
<th>Electrode Metal</th>
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<tr>
<td>Au</td>
<td>1.75</td>
<td>Pt (platinized)</td>
<td>1.47</td>
</tr>
<tr>
<td>Pt (smooth)</td>
<td>1.67</td>
<td>Co</td>
<td>1.36</td>
</tr>
<tr>
<td>Pb</td>
<td>1.53</td>
<td>Ni (smooth)</td>
<td>1.35</td>
</tr>
<tr>
<td>Fe</td>
<td>1.47</td>
<td>Ni (smooth)</td>
<td>1.28</td>
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The oxygen-hydrogen cell voltage is 1.22

As mentioned above, Fichter considers oxidation more violent at smooth platinum electrodes than at lead dioxide anodes. However, it is to be noted that in certain cases this is true only as regards the nucleus, the reverse being true of the side chain. For, Elbs \(^1\) found that at smooth platinum anodes \(p\)-nitrotoluene oxidized to \(p\)-nitrobenzyl alcohol and \(p\)-nitrocresol, while lead dioxide anodes yield \(p\)-nitrobenzoic acid. This probably has something to do with the difference in the tendency to form molecular oxygen at the two anodes. At the anode where molecular oxygen is most easily formed, the oxidation of the side chain would be expected to be most energetic, while the oxygen at the other anode would attack chiefly the nuclear hydrogens. For Fichter and Stocker \(^2\) have found that anodic, (supposedly atomic), oxygen attacks the nucleus before the side chain. This is also known to be the case with halogens. It is well to remember, when

choosing anodes for use with alternating current, that the greatest effect of the superimposed current is noticed when the metal electrode is covered with its insoluble salt. It is to be expected that the products which can be obtained at different kinds of anodes will vary somewhat due to the difference in overvoltage of various electrodes and also due to the catalytic effects of certain anode materials. Which of these two influences is the most important is a subject of debate. The relative effect of each factor varies, probably, for each new set of conditions. Iron and nickel anodes in alkaline solutions are good for oxidations only at high current densities. It is to be remembered too, that electrodes of platinum in particular, do not always behave as they are expected to. The nature of the surface, whether smooth or spongy,--whether or not the electrodes have been anodically or cathodically polarized, and the past history of the electrodes are factors which have decided influence on the course of the electrolytic processes. Prepolarization seems to convert the electrode into a very labile and active condition which is not, as yet, understood. One would expect the mildest oxidation from an electrode which had never been used as a cathode.

1. D.R.P. 297019, (1900), and 141343 (1902).
The fourth expedient is the use of salts of multivalent metals such as vanadium, cerium, manganese and, (with diaphragm only), chromium,\textsuperscript{1} which act as carriers or catalysts. These speed up absorption of oxygen by the depolarizer and increase the electrical efficiency. This expedient, according to Brockman, is most useful where the depolarizer is insoluble in the electrolyte. The oxidation is thought to proceed quite easily without the catalysts where the depolarizer is soluble.

A fifth means of controlling the intensity of the oxidation is the cooperation of the cathode,—i.e., the omission of a diaphragm. Some oxidation products from the anode are reduced partially, at the cathode. If initial reduction of the depolarizer at the cathode, and the use of a diaphragm are undesirable, excessive cathodic reduction can be avoided by the use of a cathode of small area,i.e. by having a large cathode current density which causes most of the cathodic hydrogen to escape in the molecular form. Or, instead, one may add small amounts of calcium chloride or calcium salts of resin acids, or, (in the case of neutral or slightly acid solutions), soluble chromate.\textsuperscript{2} This last forms a thin skin of chromic oxide over the cathode surface acting as a diaphragm and preventing cathodic reduction of the anode products. An alkaline solution

\textsuperscript{1} Trans. Am. Elektrochem., Soc., 40, 123, (1921).
\textsuperscript{2} Z. Elektrochem., 9, 583, (1903).
makes reduction more difficult and hence checks the effect of the cathode. The advisability of the use of a diaphragm seems to be a point of debate.\textsuperscript{1} The mechanical difficulties of clogging, hardening, decomposition, heating and increased resistance which at first made the diaphragm seem an unmitigated nuisance, have been overcome, even in the presence of tarry byproducts, by the use of the "Electrofiltros." This type of diaphragm, however, is not suitable for use in strong alkaline solutions. It is the opinion of Thachter that failure to use a diaphragm has been the cause of much trouble.

A sixth means of checking oxidation is to keep the current density below one ampere per sq. dm.,--i.e. by the use of large anodes. Electrodes in the form of concentric cylinders permit the most even distribution of current and prevent most completely excessive oxidation which would result from the piling up of current on certain parts of the electrodes. The larger the anode, the less oxygen escapes as molecular oxygen, and hence the greater the efficiency when using large currents. The large anode permits a greater quantity of depolarizer to be in contact with the evolved anodic oxygen. The greater the concentration of depolarizer,(up to a certain point), the greater is the current density which can be used without evolution of gas.

The weaker the depolarizer, (not only as regards concentration, but also as regards its reducing properties), the less must be the current density. Current concentration, i.e. ampers per litre of solution, also plays an important rôle.

A seventh means of changing or regulating the products of alternating current electrolysis has been suggested. This is the selection of the most suitable power factor. The fact that the appearance of certain lines in an arc spectrum depended upon the relation of capacity and induction in the circuit, i.e., whether or not the E.M.F. was in step with the current, led Patten to the conclusion that the percentage of alternating current available for doing work may influence the products formed.

Other factors which influence the nature and course of electrolysis in general, and which should be considered in this research are stirring, temperature, etc. Brockman and others consider stirring an absolute necessity for the successful prosecution of an electrolysis. In cases where an emulsion of the depolarizer is maintained, this factor is automatically attended to. But even where the depolarizer is in true solution the rate of electrolytic reaction is usually greater than the rate of diffusion. Hence, unless fresh quantities of the depolarizer are mechanically circulated to the anode surface, either

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excessive oxidation with loss of material or undesired evolution of oxygen with decrease in current efficiency will take place. Temperature must be regulated if too vigorous oxidation is to be prevented. For each oxidation reaction there is an optimum concentration of depolarizer for each value of current density and concentration.

Another point to be considered is that excessive and undesired decomposition of the organic starting material may be due to the formation of intermediate peroxides. If factors which discourage or prevent the formation of peroxides can be discovered, it may be possible to exclude peroxides from the solution thereby to control the extent of oxidation. Such factors as these have yet to be discovered.

It has been shown by Bircher and Harkins and others that overvoltage increases with decrease in pressure. It might be expected, then that by using large pressures, the overvoltage might be reduced to the extent that the degree of oxidation could be controlled. However, the effect of pressure is most effective at low pressures and Newbery has shown that with oxygen the overvoltage is practically constant over a range of 1-100 atmospheres.

Certain groups (such as the methyl group), when present as a substituent in the benzene nucleus are known to direct new groups towards the para (and ortho) position. Electrolytic introduction of a single hydroxyl group could be effected, probably more easily into a compound with some such substituent than into benzene itself. If this substituent could be removed then, phenol would remain. This, however, does not appear to be a very useful method.

When Tonoli 1 oxidized benzene sulphonlic acid with a very high current density at a platinum anode, he obtained phenol and sulphuric acid. In Fichter's report of this experiment 2 the value given for the current density used is 5 amps per sq. cm. This seems exceedingly high, being 500 amps per sq. dm. Stocker found that using lead peroxide anodes and .04 amps per sq. cm. the same compound yielded no phenol. It appears then that there is a possibility of obtaining phenol electrolytically and perhaps on a commercial scale, from benzene. If the process were carried on near a sulphuric acid plant, the electrolytic process would be cheapest. Benzene could be treated with oleum; the resulting solution of benzene sulphonic acid diluted would be the anolyte from which phenol and sulphuric acid would be produced. After

removal of the phenol, the resulting sulphuric acid could be used to dilute oleum, the mixture being pure enough, probably, for making fertilizers.

Electrochemical production of compounds has the advantage that mere oxidation is accomplished more economically by electrolysis than by other chemical means and that the absence of intermediate oxidizing reactants avoids expense and contamination of the products. As noted by Nernst, the proper control of electrode potential permits a wide range of gas "pressures" at the electrode surface. Further, most chemical oxidation of the aromatic nucleus if effected, results in disruption of the ring while electrochemical oxidation introduces a hydroxyl group without breaking the ring.

On the other hand, disadvantages which inhere in electrochemical methods must not be overlooked. Electrolytic methods require more room, hence larger and more expensive plants, and demand more skilled attention and greater upkeep cost than ordinary chemical plants. Further, the need of greater amount of time, thought and expenditure in the working out of the electrolytic methods and the preparation of the process for large scale production must be anticipated. Most of the difficulties encountered at first, involving diaphragms, have been overcome, as far

2. Ber. p 1562 (1897).
as acid and neutral solutions are concerned, by the use of "Electro-Filtros," \(^1\) devised by Thachter and in use since 1915.

II. Previous Work.

A search of the available literature has revealed no indication of any work having been done on the electrolysis of benzene in concentrated acetic acid solution. Segewetz and Miodon¹ used 33% acetic acid with 25% sulphuric acid as an electrolyte but even at this concentration of acid the benzene was not sufficiently soluble to make an emulsion unnecessary. Only one case has been found where electrolysis of benzene has yielded phenol and here nothing more than a rough quantitative estimate is given. Practically nothing has been reported on the use of non-aqueous solutions for electrolytic oxidations. Acetic acid of the concentration used is in a limited sense, a non-aqueous electrolyte, for without addition of sodium hydroxide, the conduction of current is negligible. Undoubtedly the current is conducted by the Na⁺ and OH⁻ ions which would not exist if there were not some water present, yet it is by reason of the large concentration of undissociated acetic acid that the depolarizer is held in true solution.

A New Emphasis as to The Mechanism of Electro-oxidation.

It has been noted in the introduction that benzene can be oxidized to phenol by the use of hydrogen peroxide, etc., as well as by anodic oxygen. In general, anodic oxidation yields the same products as do hydrogen peroxide, ozone and persulphuric acid. This fact has led some

organic electrochemists to the conclusion or belief that such electro-oxidation involves the formation of a peroxide of some sort at the anode. Hence the popularity of sulphuric acid as an electrolyte for oxidation. But Fichter has shown that products attributed to the formation of persulphuric acid etc. at the anode, can be obtained also in phosphoric acid solutions under conditions in which peracids do not form. Though the peroxide formation hypothesis has many advantages over other hypothesis, and may apply in some cases, I believe the emphasis has been put in the wrong place. Instead of considering the oxidation to proceed as a result of the formation of a peroxide, placing the emphasis of the similarity of mechanism on the peroxide, it seems to me, better to consider the oxidation a result of "nascent" or monatomic oxygen which forms supposedly, both at the anode and in the decomposition of the peroxides and thereby placing the emphasis of similarity of mechanism on the formation of the "nascent" oxygen. I have no recollection of having heard of, or of having seen in print, the emphasis so placed,—though be it granted the idea that atomic oxygen is necessary in both cases is old. But it seems that the resemblance between the chemical oxidation of peroxides and the electrical

2. Ibid. 45, p 111.
oxidation is due not to the presence of peroxides in both cases, but rather to monatomic oxygen. Instead of explaining electrolytic oxidation in terms of peroxides we can explain the mechanism of oxidation of peroxides in terms of oxidation by single molecules of oxygen as may well be the case in anodic oxidation. This does away with the necessity of hunting for peroxides in electrolytes which seem incapable of producing them.
III Experimental.

Method:

Electrodes of nickel, platinum, copper and iron were used in solutions alkaline with sodium hydroxide or acid with sulphuric acid or acetic acid. In all cases where concentrated acid was not used, the emulsion of benzene was maintained by use of a stirrer. Qualitative tests for phenol were made with samples taken from time to time, from the electrolyte. Qualitative tests for phenol were made with the various tests recorded in the literature. These are to be described in detail later. Quantitative tests or determinations of the phenol content of the reaction products were made only after separating phenol from the other oxidation products. In cases where concentrated acetic acid was used, to 40cc. of the glacial acid were added 5 cc. of concentrated sodium hydroxide solution. The desired amount of benzene when added to this dissolved readily. Cylindrical anodes, --usually of wire gauze, were used. When desired, iron or copper was electroplated previously, onto the anode.

In some cases a diaphragm was used. This was a porous cup, made of unglazed porcelain, and just large enough to contain the anode. When large currents were used, the cell was placed in cold running water to prevent volatilization of benzene etc. by the high temperature. The diagram of the cell is found in Figure 1.
**ELECTROLYTIC CELL.**

- **Anode**
- **Benzene**
- **Cooling Water**
- **Cathode**
- **Porous cup**
- **Diaphragm**
- **Anode**
- **Acetic acid**
- **Soln of Benzene**
- **Dilute NaOH solution**

**Figure 1.**
The electrical hook-up is shown in Figure 2 and 3. In Figure 2, the ohmic resistance \( R_1 \), in the AC circuit is made so large in comparison with the resistance of the cell that most of the direct current flows through the cell and relatively little through the secondary coil of the transformer. Obviously a high E.M.F. from the AC source must be used to overcome this non-inductive resistance. A choke having little ohmic resistance but allowing very little flow of alternating current was placed in the D.C. circuit to keep the AC flowing through the cell rather than through the batteries or motor generator. In Figure 3 the DC is passed through the secondary coil of the transformer. The ammeters and voltmeters are left in circuit or in parallel for only an instant at a time. As the AC voltage across the cell was small, and as the divisions on the AC instruments vary as the square of the current involved, some difficulty was experienced in obtaining an accurate measure of the alternating PD. However, this was overcome by measuring the PD between the extreme terminals of the cell and of a non inductive resistance in series with the cell; and then across the resistance alone. The PD across A-C less that across B-C equals the PD across the electrodes A-B.

Qualitative tests for phenol were made first with bromine water. The free acid was neutralized with sodium hydroxide and the solution made just acid with hydrochloric
acid. Addition of bromine water gave a yellow precipitate of tetra brom-phenol when phenol was present.

Millon's Reagent, in the presence of anything greater than 1 part of phenol in 2,000,000, gave a red coloration on standing or immediately after heating. The presence of acetate ion hindered this test and made the red coloration very transient. The reagent was prepared according to directions of Elvove as reported by H. D. Gibbs. 68g of mercury dissolved in 50 cc. of concentrated nitric acid give 40 cc. of solution to which 92 cc. of water are added. Nitric acid is added if a precipitate results and until solution becomes clear.

An equally delicate test is reported by Eykman. A few drops of alcoholic solution of ethyl nitrite added to phenol solution gave a red coloration after adding an equal volume of sulphuric acid. The test was found to give good results when alcohol, sodium nitrite and sulphuric acid were used.

There is a similar test with isoamyl nitrite. Isoamyl chloride was used with satisfactory results, the chloride being converted to the nitrite as soon as the sodium nitrite was added.

1 cc. of phenol solution with 2 cc. of concentrated sulphuric acid heated with 2 drops of benzaldehyde gave

2. J. Biol. Chem. 71. 450.
27.
a yellow to red coloration which turned to blue when diluted and made alkaline with potassium hydroxide.

A few drops of solution of hypochlorous acid, NaOCl or hypobromous acid, or NaOBr or chloride of lime, added to phenol solution with excess ammonia gave a light blue coloration.

Lactic acid and pyruvic acid used with sulphuric acid each gave the characteristic coloration with phenol.

Distinction from the polyhydric phenols was made by the use of silver nitrate. The substance tested did not reduce silver nitrate as do the polyhydric phenols. This fact, together with positive results obtained with all of the above tests, indicates quite definitely, that phenol is present in the reaction product.

Quantitative measurement of phenol content was made as follows;—First the reaction products were made alkaline with sodium hydroxide;—then just acid with hydrochloric (presence of even small amounts of acetic acid interfere with the test). The mixture was steam distilled. 150 cc of distillate were collected, or more, until the distillate showed only very slight traces of phenol. The distillate was diluted with distilled water to 250 cc. and thoroughly mixed. The odor of phenol was quite obvious. A standard solution of phenol containing .0002344g. per cc. was prepared. Equal volumes of this and the solution of unknown were placed in small homemade comparison tubes and to these, equal volumes of bromine
water were added. Distilled water was added to the one which was most cloudy until the two were equally cloudy. This comparison, made by looking through the tubes into diffuse light, gave accurate results provided the test was made speedily, i.e. before coagulation of the precipitate took place. From the volume of liquid in each tube, the concentration of phenol in the unknown solution could be calculated from the known concentration of the standard.

Steam distillation of the reaction mixture provided the only successful means of separating phenol from the other products. Preferential adsorption of phenol on charcoal was tried, but the concentration of phenol was so low that the scheme did not meet with success.

In some experiments a rectified current was used. A tantalum metal electrode was the rectifier. A small sheet of tantalum was placed in the cell and 90 volts DC were applied for 2 hours. The large fall of potential at the surface of the rectifier caused sparking at the electrode. After 2 hours only 0.04 amperes were flowing. The tantalum became covered with a blue deposit. A PD of 9 volts was applied for 18 hours, after which time no current was flowing which was large enough to be measured on the ammeter. This electrode was then used as a rectifier. The cell was placed in an AC circuit taken from a transformer. 15.2 volts AC gave a current which measured 60 milliamperes.
on a DC milliammeter.

In some experiments the direction of flow of current was reversed in the middle of the electrolysis in the hope that oxidation of reduction products might yield some compounds different from those obtained by oxidation of the original reactant. These experiments involved the use of a number of different types of current. If the ordinary direct current be represented by (a) in Figure 4, the ordinate indicating the extent of the charge on the electrode and the abscissa indicating time, then (b) represents the nature of the current when current is reversed. The alternating current used by itself is represented by (c), and when used superimposed upon direct current, by (d) and (e). That obtained with the tantalum electrode is represented by (f). The decomposition potential indicated by the red lines need not necessarily have the same value above as below the line of zero voltage (green). The actual value for the decomposition potential was not obtained. Attempts were made to measure it in a solution of 50 cc. of benzene in 100 cc. of acetic acid, but there was no sudden change of slope of the voltage-current graph, as can be seen by the results recorded in the next section; hence there seemed to be no particular value for the decomposition potential over the range studied.
CURRENT GRAPHS.

(a) Simple Direct Current.

(b) Direct Current Reversed.

(c) Simple Alternating Current.

(d) Alternating current superimposed on Direct.

(e) Alternating current superimposed on a larger Direct Current.

(f) Intermittent current obtained with the Tantalum electrodes.

Green Lines; --Zero voltage.
Red Lines; --Decomposition potentials.
Black Lines; --Variation of charge on electrodes or voltage.
Ordinates; --Voltage or charge.
Abscissae; --Time.

Figure 4.
IV. Results.

The current-voltage curve for a solution of 50 cc. benzene in 100 cc. acetic acid is given in Graph I. Where alcohol is substituted for acetic acid, the results shown by Graph II, acid (with HCl) solution and by Graph III, basic (with NaOH) solution, are obtained. In the case of the last two graphs there seems to be a decomposition at about 1.6 volts but with benzene in acetic acid the curve is quite smooth.

The only other products examined closely were those obtained from experiments 1, 2, and 17. After the products from Experiment 2 had stood for some time (about 2 months), many crystals separated out. These were separated from the mother liquor and washed with ether, in which they are insoluble, dried between towels and desiccated over anhydrous calcium chloride for two weeks. Four samples were weighed out into desiccated, weighed crucibles. After heating for 12 hours at 104°C, they were reweighed and again after 1 hour at 150°C. All four weights check closely with what is to be expected from the decomposition of sodium acetate with two molecules of acetic acid of crystallization to the ordinary anhydrous sodium acetate. Treatment with excess hydrochloric acid followed by drying and reweighing gave a result which checked closely with that expected from the conversion of sodium acetate to sodium chloride. The crystals dissolved
Solution 50 c.c. of Benzene in H.OAc (1:100).
10 NoH till ready to precipitate the Benzene.

Direct current without application
of Alterating Current.

Graph I.

Platinum electrodes.
Porous Cup.
Benzene 50cc in 6% EtOH, 125cc.
+ NaOH solution

Direct current only

Graph III.
readily in water giving an acid solution. Sodium hydroxide solution was standardized and used to titrate a known weight of the crystals. From the normality of the solution and the volume used, the titration equivalent was found to be 101.6. From this data it was evident that the crystalline substance was \( \text{CH}_3\text{COONa} \cdot \text{CH}_3\text{COOH} \). This substance has been recognized for some time, and of course, is not a product of electrolysis. But crystals obtained in a similar manner from Experiment 1 failed to correspond to any previously recognized compound.

These crystals, were, like those of Experiment 2, insoluble in ether, carbon tetrachloride, only slightly soluble in 95% ethyl alcohol, but very soluble in water. Their aqueous solution was acid. However, titration showed an equivalent weight of 151. Obviously this is not the same compound as was obtained in Experiment 2 though both compounds have much in common. Both gave off, on heating, an inflammable gas, (acetone in the case of Experiment 2), and left a residue of sodium carbonate. Addition of concentrated sulphuric acid to fresh crystals liberated an acid which, in smell, resembled closely, acetic acid. Hence the compound in question appears to be the sodium salt of an organic acid together with acetic acid of crystallization. When the crystals are heated gently, it seems that the acetate radical unites with the sodium ion giving sodium acetate, excess acids being driven off. The
residue melts at 312°C as does sodium acetate. Vacuum distillation of these crystals together with sulphuric acid, yielded a trace of liquid which had the smell of acetic acid, but unmistakably, also that of vanilla. Just how vanillin was formed from benzene, is uncertain, but it is possible that isoeugenol was an intermediate product as vanillin has been obtained from isoeugenol electrolytically. The melting point of the crystals was 91-94°C and decomposition took place at 160-162°C.

The unknown organic acid radical of this compound may be the same as that obtained in a different manner in Experiment 17. After all the acid has been neutralized with sodium hydroxide and the phenol had been steam distilled the residue was allowed to cool. Crystals 8 x 1 x 1 cm. formed. These treated in a manner similar to the treatment described above for crystals from Experiment 2, proved to be something other than pure hydrated sodium acetate, though both sodium and acetate radicals were involved in the make up of this crystalline substance.

The yields of phenol and the conditions under which they were obtained in the various experiments are indicated in the following table.

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<td>Amps</td>
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<td></td>
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<tr>
<td>E.</td>
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<td>.0192</td>
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<td>.05</td>
<td>7.4</td>
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<tr>
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</tr>
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<td>&quot;</td>
</tr>
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<td>48</td>
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<td>11.</td>
<td>1.4</td>
<td>9</td>
<td>5days nichr&quot;</td>
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<tr>
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<td>10</td>
<td>3days Cu no&quot;</td>
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<tr>
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<td>&quot;</td>
<td>&quot;</td>
<td>4</td>
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<td>Cu</td>
</tr>
<tr>
<td>19.</td>
<td>2</td>
<td></td>
<td>24hour</td>
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<tr>
<td>20.</td>
<td>4</td>
<td></td>
<td>8</td>
<td>&quot;</td>
</tr>
<tr>
<td>21 &amp; 24</td>
<td>&quot;</td>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>23.</td>
<td>30 &amp; up</td>
<td></td>
<td>5½</td>
<td>&quot;</td>
</tr>
<tr>
<td>24.</td>
<td>40 &amp; up</td>
<td></td>
<td>1½</td>
<td>Pt</td>
</tr>
<tr>
<td>25.</td>
<td>15-40</td>
<td></td>
<td>2½</td>
<td>&quot;</td>
</tr>
<tr>
<td>26.</td>
<td>10-30</td>
<td></td>
<td>1</td>
<td>&quot;</td>
</tr>
<tr>
<td>27.</td>
<td>15-30</td>
<td></td>
<td>1½</td>
<td>Cu</td>
</tr>
<tr>
<td>28.</td>
<td>19-40</td>
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<td>Pt</td>
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<td>29.</td>
<td>19-40</td>
<td></td>
<td>1½</td>
<td>&quot;</td>
</tr>
<tr>
<td>30.</td>
<td>20</td>
<td></td>
<td>1</td>
<td>&quot;</td>
</tr>
<tr>
<td>31.</td>
<td>15-20</td>
<td></td>
<td>1½</td>
<td>&quot;</td>
</tr>
<tr>
<td>32.</td>
<td>20-22</td>
<td>1.8</td>
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<tr>
<td>33.</td>
<td>20-27</td>
<td>1.9</td>
<td>1½</td>
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</table>
V. Discussion of Results.

As can be seen from the results, the superimposition of alternating current on direct current does not seem to increase the yield of phenol. Further, the higher the voltage used, (within reasonable limits), the more phenol is obtained. This corresponds to the findings of Stocker and Tonoli when they electrolized benzene sulphonic acid. It will be noted too, that the presence of a small quantity of the salts of copper, iron or vanadium increases the yield of phenol, but that a large quantity of any of these salts (especially of the first two), causes excess oxidation.

VI. Summary.

1. A fairly detailed summation of the factors which influence electrolyses such as this one, has been made.

2. Phenol has been obtained electrolytically from benzene; the highest yield, however, was 1.76%.

3. This has, together with the results of Fichter and Uhl referred to on page 2, provided evidence favoring the prediction of several organic electrochemists that phenol is the first electro-oxidation product of benzene.

4. In these experiments, the superimposition of Alternating Current did not increase the yield of phenol.

5. The presence in the electrolyte, of small quantities of salts of multivalent metals is beneficial.

6. A high voltage is preferable to a low voltage if phenol is desired.

7. Electrolysis of benzene produces an equilibrium mixture of phenol with other products. The concentration of phenol was probably never more than 2% of the original concentration of benzene.
In conclusion I wish to express my thanks to Dr. R. H. Clark for kindly criticism and the many helpful suggestions offered throughout these investigations.
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THE

EFFECT OF CERTAIN CHEMICALS ON THE HYDROLYTIC

ACTIVITY OF RICINUS AND PORK PANCREAS LIPASE.
THE EFFECT OF CERTAIN CHEMICALS ON THE HYDROLYTIC ACTIVITY OF RICINUS AND PORK PANCREAS LIPASE.

Following up the work done in these laboratories on the activation of amylase, experiments were carried out to discover the influence of certain chemicals, especially organic chemicals, on the hydrolytic activity of lipase. 1 Certain chemicals have been found capable of shortening the dormant period of seeds. The above mentioned work has shown that this may be due to the activation of amylase in the seed, resulting in the production of a hexose which serves as a source of food for the growing embryo. In the case of certain seeds, it may be that the dormant period may be broken prematurely, by the use of certain chemicals which activate lipase. Lipase is known to be present in an active form in germinating endosperms of oily seeds, but not in the resting seeds. Furthermore, lipase occurs in many different forms within the animal body. Serious cases of tuberculosis are usually accompanied by low values of serum lipase. The lipase inhibits the growth of the tubercle bacillus by destroying the waxy or fatty covering which is characteristic of Mycobacterium tuberculosis. If an activator for serum lipase could be found, injection of this activator, into the blood stream, might result in

helping the body to win the battle against the tubercle bacillus.

Previous Work:

Much work has already been done along this line. The results are very conflicting. Palmer reports that with commercial lipase (source not stated), formaldehyde in concentrations between 1 part in 1000 and 1 part in 1500 gave a 16% increase in activity. Mercuric chloride in concentrations .1 to .3% completely inhibited the hydrolysis. Acetone .6% to 12% inhibits by 11%-24%. Iodine .09% retards the rate 96% and Bromine .25% retards activity by 93.2%. Hydrochloric acid has been reported as an activator.

Enzymes Used.

In these studies, two lipases were used. One was animal in source. This was supplied by Eimer & Amend. It was prepared from pork pancreas. The pancreas was vacuum dried at a low temperature, and defatted with benzol. Benzol was removed in vacuo. The product was then powdered and sifted. The other lipase was a plant lipase. Castor oil beans, (Ricinus Zanzibariensis, mixed), were germinated by keeping the seeds in a moist condition at 80°F for one week. The seeds were shelled by hand and the endosperms were coarsely ground and washed once with ether. This material

was extracted in a Soxhlet with ethyl ether for one week. The oil free material was air dried and then placed in a vacuum desiccator over calcium chloride for three weeks. This dried product was powdered in a mortar and passed through a 100 mesh sieve.

Preparation of Substrate.

Cold drawn castor oil was the substrate used. A suspension of the oil was made with gum acacia. 0.55 grams of powdered gum acacia was mixed thoroughly in a mortar with 90 cc. of castor oil, till an oily paste resulted. Water was added in 5 cc. quantities with thorough mixing between each addition. After about 100 cc of water has been added, the mixture was diluted to 2500 cc. This gives a stable emulsion of 3.2% oil in water. The emulsions prepared in this way were found to be much superior to those prepared according to directions in the literature where the gum acacia is hydrated before addition of the oil.

Method.

75 cc. portions of this castor oil milk were placed in 125 cc. Erlenmeyer flasks. The flasks were previously treated to remove traces of di- or multi-valent salts such as mercuric salts, which might be on the surfaces of the glass. 3 grams of lipase were suspended in 100 cc of this "milk" and 3 cc. of the suspension was added to the 75 cc. portions of "milk". The chemicals were then added, except to the controls, and all flasks were incubated at 37°C. Titrations were made on 25 cc. portions from 24 to
96 hours later. The extent of hydrolysis was ascertained by titration of the fatty acids produced, with 0.1 Normal sodium hydroxide solution, using a deep red of phenolphthalein as an end point. A burette of small diameter permitted accurate readings of small volumes of sodium hydroxide solution. All titration values were compared with those obtained from (1) enzyme plus substrate without chemicals added, (2) substrate plus chemical without enzyme added, (3) substrate in water without either enzyme or chemical.

**Results.**

<table>
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<th>Effect on Ricinus lipase</th>
<th>Effect on Pancreas lipase</th>
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</thead>
<tbody>
<tr>
<td>Hydrochloric acid .023%</td>
<td>decreases activity</td>
<td>decreases activity</td>
</tr>
<tr>
<td>Lactic acid .2%</td>
<td>almost complete inhibition</td>
<td>almost complete inhibition</td>
</tr>
<tr>
<td>Iodine aqueous .0019%</td>
<td>marked inhibition for first 24 hours followed by a 20% increase over controls after 96 hours.</td>
<td></td>
</tr>
<tr>
<td>Bromine aqueous .125%</td>
<td>same action as for iodine.</td>
<td></td>
</tr>
<tr>
<td>Acetone 5% and down</td>
<td>inhibits 80% and down</td>
<td>inhibits 60% down.</td>
</tr>
<tr>
<td>Ethyl benzyl ketone .2%</td>
<td>inhibits 40%</td>
<td>inhibits 40%</td>
</tr>
<tr>
<td>Isobutyl cresol ketone .2%</td>
<td>inhibits 5% and less</td>
<td>inhibits 5% and less.</td>
</tr>
<tr>
<td>Methyl isobutyl cresol ketone .2%</td>
<td>Effect small, sometimes accelerates by 5%.</td>
<td>Sometimes accelerates by 5%.</td>
</tr>
<tr>
<td>Chemical and Concentration</td>
<td>Effect on Ricinus lipase</td>
<td>Effect on Pancreas lipase</td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Formaldehyde Technical .1%</td>
<td>inhibition 80%</td>
<td>inhibition 30%</td>
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<tr>
<td>Cinnamic aldehyde .005-.1%</td>
<td>marked decrease in both cases.</td>
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<td>Hydrocinnamic aldehyde .002-.05%</td>
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<td>decrease</td>
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<tr>
<td>Crotonic Aldehyde .002%-.05%</td>
<td>complete to 10% inhibition</td>
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</tr>
<tr>
<td>Acrylic aldehyde .02%</td>
<td>complete inhibition</td>
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<tr>
<td>Acetaldehyde .02%</td>
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<td>Paraldehyde .02%</td>
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<td>slight decrease</td>
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<tr>
<td>Furfural .002%-.05%</td>
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<tr>
<td>Iso butyl Aldehyde .002%-.05%</td>
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<td>Salicylic aldehyde .002%-.05%</td>
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<td>Mercuric chloride .01%</td>
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<td>.003%</td>
<td>90%</td>
<td>80%</td>
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<td>Potassium thiocyanate 1% fresh solution</td>
<td>120% increase</td>
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<td>old solution</td>
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<td>fresh from different source</td>
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Chemical and Effect on Ricinus Effect on Pancreas
Concentration. lipase lipase.

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<tbody>
<tr>
<td>Ethylene chlor-hydridn .3%</td>
<td>decrease</td>
<td>decrease</td>
</tr>
<tr>
<td>Thiourea .15%</td>
<td>decrease</td>
<td>decrease</td>
</tr>
<tr>
<td>Cystein 1/15 conc of sat. solution</td>
<td>decrease</td>
<td>decrease</td>
</tr>
<tr>
<td>Skatol (Sat) 1--1/5 saturated</td>
<td>decrease</td>
<td>decrease</td>
</tr>
<tr>
<td>Pyridene .05%--.002%</td>
<td>increase in low concentrations.</td>
<td></td>
</tr>
</tbody>
</table>

A compound with the formula

\[
\begin{align*}
H-N-----C=O \\
C_2H_5-S-C=O \quad C-\text{CH}_3 \\
\end{align*}
\]

in concentrations of .02% gave decreases in both cases.

Conclusions.

It will be observed, that as a whole aldehydes cause a decrease in activity in the concentrations used. Ketones likewise appear to decrease the activity. The double bond in an aldehyde seems to have an appreciable effect. Potassium thiocyanate decreases the activity of animal lipase, but sometimes increases the activity of Ricinus lipase. This may be due to the action of the K⁺ ion rather than to the SCN⁻ ion. Old solutions seem to contain some decomposition product of KSCN which inhibits activity. Further, different samples of the material have different effects indicating that the C.P. material contains varying...
quantities of impurity. Hydrochloric in the concentrations found by Palmer to produce activation (not using an emulsion) did not cause an activation with the suspension. Lactic acid is considered by some as that substance which converts the zymogen to lipase in plants' seeds. However in vitro almost complete inhibition results with even reasonably small concentrations. The results obtained with the free halogens are very interesting. It will be observed that much larger quantities of bromine are required to give the same effect as small quantities of iodine.