TABLE III (*Concluded*)

	Formula	M. p., °C.	Yield, %	Nitrogen, analyses, % Calcd.	Found
1	$C_4H_9CO_2HNC_6H_4OCOC_6H_5$	62.5	63	4.47	4.54
2	$C_4H_9CO_2HNC_6H_4OCOC_6H_5$	85.5–85.8	60	4.47	4.35
3	$C_6H_5COHNC_6H_4OCO_2CH_3$	128	78	5.17	5.07
4	$C_4H_9COHNC_6H_4OCOC_6H_5$	73–74	45	4.71	4.75
5	$C_6H_5COHNC_6H_4OCOC_4H_9$	103.5–104.5	36	4.71	4.73
6	$C_4H_9COHNC_6H_4OCOC_6H_5$	96–97.5	29	4.71	4.69
7	$C_6H_5COHNC_6H_4OCOC_4H_9$	113.5–117	35	4.71	4.57

Summary

1. A study has been made of the diacyl derivatives of *o*-aminophenol, using the benzoyl group against a series of carbalkoxy groups. It is found that the carbalkoxy group is able in most cases to displace the benzoyl group from the nitrogen. Where, however, there is a large difference in the weights of the two groups, as in the case of carbomethoxy (CH_3OCO) and the benzoyl (C_6H_5CO) groups, the benzoyl group is able to displace the lighter group completely, though the reverse takes place when the next heavier (C_2H_5OCO) group is used.

2. No evidence of differences in the action of *normal* and *iso*-acyl groups when used against the benzoyl group is observed in the case of the *n*- and *iso*carbobutoxy (C_4H_9OCO) groups and of the *n*- and *iso*valeryl (C_4H_9CO) groups.

3. New mono- and diacyl derivatives of *o*-aminophenol have been prepared and studied.

LAFAYETTE, INDIANA

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF OREGON]

THE EFFECT OF VARIOUS PREPARATIONS ON THE GROWTH OF BAKERS' AND BREWERS' YEASTS

BY ROGER J. WILLIAMS, MARION E. WARNER AND RICHARD R. ROEHM

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In a previous paper[1] the fact was emphasized that different strains of yeast, all of which are called by the name *Saccharomyces cerevisiae*, may react quite differently toward nutrilites.[2]

Since this article was written considerable progress has been made in this Laboratory in concentrating the active material from yeast and other sources, the purification of which was the main object of our research. Before carrying the purification further we were interested to know how important the substance in question might be; whether, for example, it functions for one strain of yeast only or whether it might not be important in the nutrition of several strains of yeast. A second reason for making

[1] Williams, Wilson and Von der Ahe, THIS JOURNAL, **49**, 227 (1927).

[2] See Williams, *Science*, **67**, 607 (1928).

this study, in addition to our interest in the significance of our own preparation, was that few controlled experiments have been reported in which different strains of yeast have been compared directly with each other and we deemed it important to know just how wide a variation might be expected.

The principal purpose of this paper, therefore, is to give results of experiments in which six strains of yeast (three bakers' and three brewers' yeasts) were compared with each other in their behavior toward various preparations, including the "α-Bios" and "β-Bios" of Eddy and Kerr,[3] "Bios I" (Inositol) of Lash Miller and our own concentrated material, as well as cruder preparations.

In Figs. 1–6 are given results in which crude yeast extract, prepared essentially according to the method outlined in the paper previously cited, was introduced in various amounts into our synthetic media and the growth of the different yeasts tested in the resulting solutions. The technique used was essentially that described previously, with a few minor modifications. Ten cubic centimeters of medium was used instead of 50. To it is added 1 cc. of a solution containing the material to be tested, plus 1 cc. of a yeast suspension (in sterile medium) of such concentration that the average for each large square on a Levy counting chamber was $3^1/_2$ to 4 cells. In these and other experiments in which a yeast crop was too small to weigh, the crop was estimated by visual comparison with suspensions containing known amounts of yeast. Neither this method nor that of weighing very small crops is highly accurate but they are sufficiently so for the purpose involved. In no case do we draw conclusions from small differences. In the experiments already mentioned, two sets of experiments were run in each case; one set was allowed to grow for eighteen hours and the other set for forty-eight hours.

The pure cultures of yeasts used in these tests were obtained from the following sources: Numbers 578 and 2335 from the American Type Culture Collection, Old Process Bakers' Yeast and Gebrüde Mayer from the Fleischmann Company, Ruppert Brewing Yeast and Untergärige Hefe K from Dr. W. H. Eddy. "Old Process Bakers' Yeast," "Gebrüde Mayer" and No. 2335 are bakers' yeasts and the others are, according to our best information, brewers' yeasts. We have been a little in doubt with regard to yeast No. 578, which has been used in this Laboratory most extensively in recent years. This culture originated in the collection of F. W. Tanner of the University of Illinois, which was handled by the American Type Culture Collection. It was first obtained with the understanding that it was bakers' yeast. In the late catalog of the American Type Culture Collection, however, it is listed as a brewers' yeast.

[3] See Peskett, *Proc. Soc. Exptl. Biol. Med.*, **25**, 340 (1928).

Although the various yeasts grow at different rates and show other peculiarities, the following general observations may be made.

1. The growth of all the yeasts tested is remarkably stimulated by the addition of yeast extract to our synthetic medium. In all cases the maximum yeast crops were obtained when the largest amounts of extract were added.

2. When the yeast was allowed to grow for as long as forty-eight hours, there was a striking contrast between the control cultures and those in which very small amounts of extract were added.

3. When the yeast was allowed to grow for only eighteen hours, this contrast was less marked, yet the eighteen-hour growth curves are, in general, much more regular, approximating a straight line in the lower concentrations.

We believe that these results materially strengthen the suggestion previously made that a short growth period is superior to longer growth periods for quantitative measurements of dosage. Accordingly the rest of the experiments reported in this paper were carried out using the eighteen-hour growth period.

In Table I are given results of experiments in which the six yeasts are tested on two of our preparations, each added separately and both added together. Results reported previously showed that for yeast No. 578 two complementary preparations could be obtained. One fraction is adsorbed by fuller's earth; the other is not. Both together are necessary to stimulate appreciably the growth of this yeast. The preparations used in this experiment were (1) the crude unadsorbed residue obtained by treating the yeast extract with fuller's earth and evaporating the filtrate to dryness, (2) a highly concentrated material (Z_2) derived from the fraction which *is* adsorbed by fuller's earth from yeast extract.

The method of preparation of "Z_2" will not be described in great detail since we do not claim that the material is pure in any sense, and because we have subsequently materially modified our procedure for concentrating such material. Its preparation involved the following steps: (1) The "activated" fuller's earth was extracted with baryta, the baryta removed by sulfuric acid and the filtrate evaporated to dryness. (2) The residue was fractionated with the following solvents: 80% alcohol, water and methanol. In this process the activity was concentrated to approximately one-fifth of its original weight by applying the solvents in the order indicated and rejecting the insoluble material in each case. The Z_2 preparation is highly soluble in water. (3) The remaining soluble material was fractionated, following the method of Jansen and Donath,[4] the Z_2 coming from the fraction precipitated between the P_H values 6.5

[4] B. C. P. Jansen and W. F. Donath, *Nededeel. Dienst Volksgezondheid Nederland, Indië*, Part I, 86–99 (1927).

and 7.5. Other fractions, however, also contained much activity. (4) The silver precipitate was decomposed with hydrochloric acid and carefully evaporated to dryness. The gummy residue constitutes "Z_2."

TABLE I[a]

EFFECT OF UNADSORBED RESIDUE AND Z_2 CONCENTRATE ON SIX YEASTS

Yeast	Blank	8 Mg. of U. R.	0.005 Mg. of Z_2	8 Mg. of U. R. +0.005 Mg. of Z_2
578	0.2	0.5	0.3	6.5
G. M.	.6	9.7	1.6	12.1
O. P.	.2	0.5	0.3	6.2
U. H. K.	.1	0.2	.3	1.1
2335	.1	3.5	.1	2.7
Ruppert	.15	1.2	.5	2.1

[a] In this and following tables "U. R." stands for the unadsorbed residue and "Z_2" for our concentrate, both of which are previously described. The numbers represent the yeast crops expressed in milligrams obtained in eighteen hours. The blank is our synthetic medium unless otherwise indicated.

It will be noted in this experiment that in the cases of Yeast 578, O. P. yeast, and Untergärige Hefe K, there is a marked complementary action of the two preparations. In Gebrüde Mayer and Ruppert yeast the effect of the two preparations is approximately additive, while in the case of Yeast 2335 the concentrate seems to have no stimulating effect. (However, in two other experiments this preparation did have a slight stimulating effect on Yeast 2335.)

In the cases of Yeast 2335 and Gebrüde Mayer the unadsorbed residue alone is very effective. Experimenters who might be working with either of these yeasts would naturally conclude that fuller's earth treatment removes practically none of the active substance from an active solution, whereas exactly contrary results are obtained with three other yeasts.

Table II depicts experiments in which were tested, in parallel, the effects of 1-mg. doses each of our unadsorbed residue, Eddy and Kerr's "α-Bios" and "β-Bios." The latter two preparations were kindly furnished by Drs. Eddy and Kerr.

TABLE II

EFFECT OF UNADSORBED RESIDUE, "α-BIOS" AND "β-BIOS" ON SIX YEASTS

Yeast	Blank	1 Mg. of U. R.	1 Mg. of "α-Bios"	1 Mg. of "β-Bios"
578	0.1	0.5	0.1	0.4
G. M.	3.7	6.5	2.9	5.0
O. P.	0.1	0.5	0.1	0.2
U. H. K.	.15	.5	.2	.15
2335	.025	.3	.025	.025
Ruppert	.1	.3	.1	.1

In no case does the "α-Bios" appear to show distinctly stimulating effect on the yeasts when tested in this way. The "β-Bios" shows appreciable stimulation in the case of two yeasts, but in each case an equal

dosage of unadsorbed residue gives a greater stimulation. It may be remarked here that Eddy and Kerr have, in their experiments, generally used a longer growth period in which case *very small* amounts of active

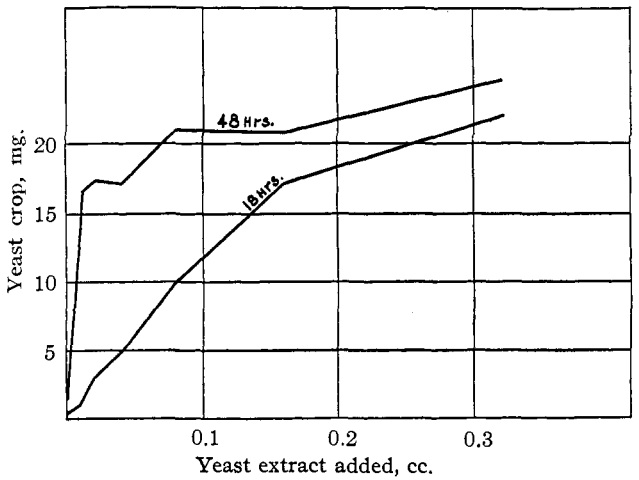

Fig. 1.—Yeast No. 578.

material may cause very marked increases in the yeast crop. This effect of the longer growth period is shown very clearly in the contrast between the upper and lower curves in Figs. 1 and 2.

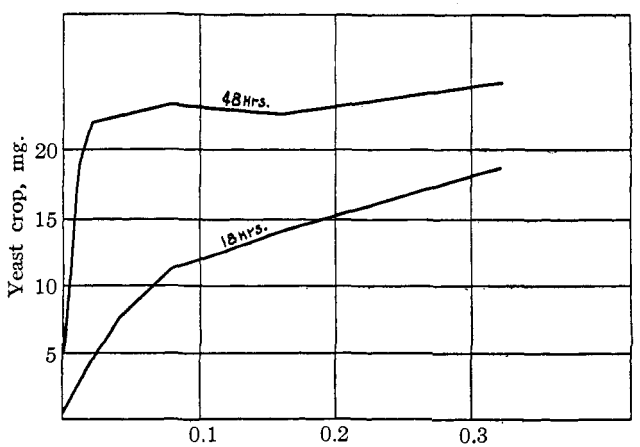

Fig. 2.—Gebrüde Mayer.

In Table III are given results of tests of "α-Bios" and "β-Bios" in conjunction with our concentrate, which we have designated as "Z_2." The yeast used in this experiment was No. 578 which, it will be noted

in Table I, is very slightly affected by "Z_2" alone. The purpose was to see whether either of these preparations acted as a supplement to the preparation which we obtained from material adsorbed on fuller's earth. Both "α-Bios" and "β-Bios" have been obtained from material which was not adsorbed by fuller's earth. The results of the tests are negative, that is, neither "α-Bios" nor "β-Bios" appears to supplement "Z_2" so far as yeast No. 578 is concerned.

TABLE III

EFFECT OF "α-BIOS" AND "β-BIOS" PLUS "Z_2" ON YEAST 578

Blank	0.4	0.1 mg. of "α-Bios"	0.4
1 mg. of "α-Bios"	.2	0.1 mg. of "α-Bios" +0.005 of Z_2	.4
1 mg. of "α-Bios" +0.005 of Z_2	.3	0.1 mg. of "β-Bios"	.5
1 mg. of "β-Bios"	.4	0.1 mg. of "β-Bios" +0.005 of Z_2	.5
1 mg. of "β-Bios" +0.005 of Z_2	.6		

In the experiment referred to in Table IV, yeast No. 578 was used. The amounts of "Z_2" and unadsorbed residue in the media were each varied separately, with the results indicated. It is apparent that if either component is held at a proper level, variation of the other component causes a variation of yeast crop which is roughly proportional in the lower concentrations to the amount of the preparation added. It is also apparent from the results that a dosage of "Z_2" as low as 0.0006 mg. (0.00005 mg. per cc.) causes marked stimulation in the growth of the yeast. If a longer growth period is used this same dosage will cause a much greater contrast between the crop obtained and that obtained in the control medium. Results of this sort have been obtained repeatedly. In Fig. 1 a case appears in which a dosage which causes approximately a doubling of growth in eighteen hours, causes a 33-fold increase over the blank when the forty-eight hour growths are compared.

TABLE IV

EFFECT OF VARYING "Z_2" AND UNADSORBED RESIDUE (YEAST 578)

Blank (8 mg. of U. R.)	0.6	Blank (0.0024 mg. of Z_2)	0.2
Same + 0.0006 mg. of Z_2	1.4	Same + 1 mg. of U. R.	.5
Same + 0.0012 mg. of Z_2	2.2	Same + 2 mg. of U. R.	.7
Same + 0.0024 mg. of Z_2	3.3	Same + 4 mg. of U. R.	1.8
Same + 0.0048 mg. of Z_2	5.6	Same + 8 mg. of U. R.	3.3
Same + 0.0096 mg. of Z_2	5.5	Same + 16 mg. of U. R.	3.9
Same + 0.096 mg. of Z_2	11.8	Same + 32 mg. of U. R.	4.3

The results of tests of Bios I (Inositol), discovery of which was announced[5] from Lash Miller's laboratory are shown separately in Table V because the other work had been completed before these publications appeared. The inositol used in these experiments was obtained from the Eastman Laboratories. It will be noted that in the doses used the inositol

[5] *Science Suppl.*, X, July 6, 1928; Eastcott, *J. Phys. Chem.*, **32**, 1094 (1928).

has very little effect on the growth of any of the six yeasts either alone or in conjunction with our "Z_2" concentrate.

It may be that our "Z_2" is not the proper supplement to the inositol. However, since "Z_2" does supplement our "unadsorbed residue," inositol

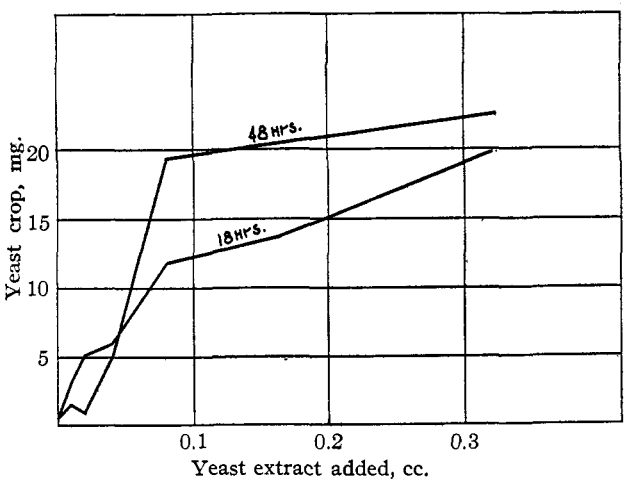

Fig. 3.—Old Process Bakers' Yeast.

is evidently not the active substance in this residue. From the method of preparation of "Z_2" and the very small doses which are effective, it is clear that inositol is not the active material in the "Z_2" concentrate.

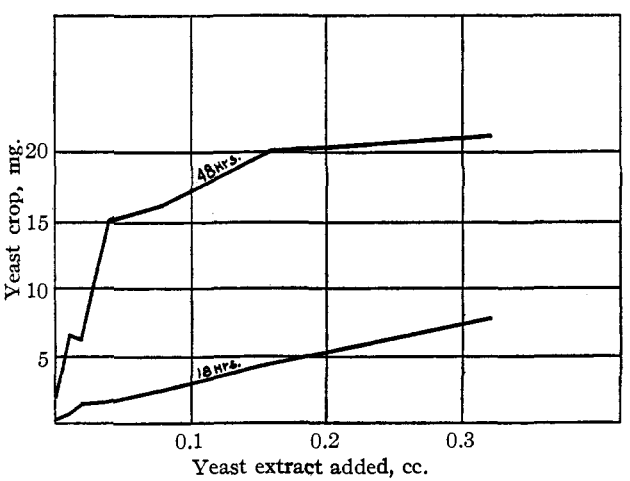

Fig. 4.—Untergärige Hefe K.

The conclusion may be drawn, therefore, that inositol does not constitute the active material in either of the two supplementary fractions which

we have found to be, when used together, very stimulatory to the growth of certain yeasts.

An examination of Miss Eastcott's evidence as to the functioning of inositol in yeast growth is not reassuring. We have no reason to doubt the approximate homogeneity of the product which was obtained, but evidence seems to be lacking both as to its potency and constancy. Relatively large doses of inositol were required to cause an effect. A dosage of 0.0033 mg. of inositol per cc. is required to about treble the yeast crop which is obtained in eighteen hours when "Bios II" alone is present. By comparison with our results it may be noted in Table IV that 0.0001 mg. of "Z_2" per cc. (0.0012 mg. per 12 cc.) more than trebles the growth

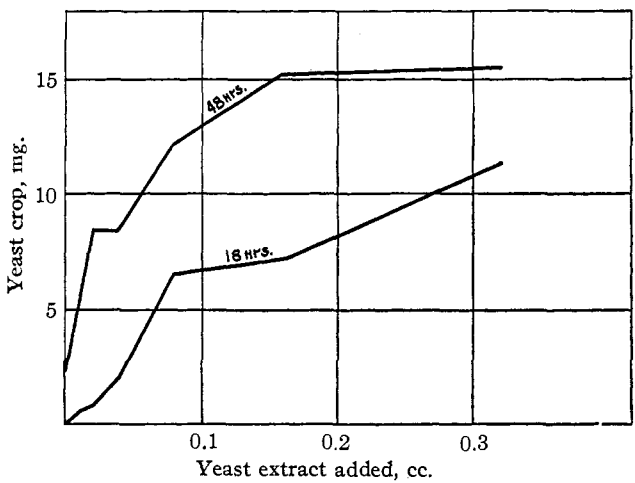

Fig. 5.—Yeast No. 2335.

in eighteen hours. Yet our "Z_2" is obviously impure and recent work has indicated that it consists for the most part of inert material.

As to the constancy of the activity of inositol Miss Eastcott gives no evidence. She states that Kahlbaum's inositol and that obtained from tea showed "no difference" in behavior but no data are given to support the statement. Neither is evidence given nor even a statement made that the activity of the inositol remained constant after recrystallization. In the discussion the question as to the identity of her product with inositol is thoroughly treated but no emphasis is placed upon the question of the identity of her product with the physiologically potent substance for which she was searching.

The inositol that is on the market is obtained from a vegetable source which is probably rich in "bios," and it may be difficult to free the inositol from traces of these impurities. Even when purified on an enormous scale and in a very efficient manner, cane sugar has repeatedly been shown

to contain "bios." We have also observed the occurrence of "bios" in highly purified asparagine and Kahlbaum's lactose.

The fact that the yeast crops recorded in Table V are very low as compared with the others is due, we feel sure, to the physiological state of the yeast used. This experiment was made early in the fall after a summer vacation during which the yeast cultures were not transplanted regularly.

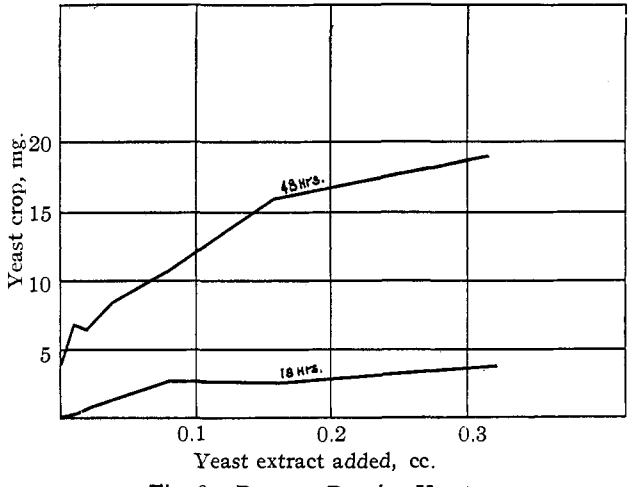

Fig. 6.—Ruppert Brewing Yeast.

After having transplanted the cultures a number of times the growths increased and in a few weeks came back and have remained at the higher level. There is no evidence that these slower-growing cultures of yeast reacted materially differently toward the preparations than the yeast used in the earlier experiments.

TABLE V

EFFECT OF "BIOS I" (INOSITOL) ON SIX YEASTS

		Inositol						
Yeast	Blank	1 mg.	0.01 mg.	1 mg. +0.0024 mg. of Z_2	0.01 mg. +0.0024 mg. of Z_2	0.0024 mg. of B_2	8 mg. of U. R. +0.0024 mg. of Z_2	8 mg. of U. R.
578	0.05	0.05	0.05	0.1	0.075	0.075	0.7	0.15
G. M.	.4	.4	.4	.4	.4	.4	3.6	3.9
O. P.	.025	.05	.025	.1	.1	.1	0.8	0.15
U. H. K.	.025	.075	.025	.075	.05	.05	0.55	0.45
2335	.025	.025	.025	.025	.025	.025	6.0	4.4
Ruppert	.025	.05	.05	.05	.05	.075	0.7	0.5

Our thanks are due the Research Committee of the University of Oregon for its support of this work and to the Fleischmann Company for the fellowship which they have granted and under which a portion of this work was done.

Summary

1. All six yeasts tested show a very marked stimulation of growth when varying quantities of yeast extracts are added to a synthetic medium.

2. All yeasts fail to show an "optimum" concentration of yeast extract either when an eighteen-hour or a forty-eight-hour growth period is used. That is, in every case the largest crops were obtained when the most extract was added. When toxic materials are present in the extract these results may not be obtained.

3. In the case of most, if not all six, of the yeasts studied it appears that a short growth period (eighteen hours) is much more easily adapted than a longer growth period (forty-eight hours or more) for quantitative studies on yeast nutrilites. If a long growth period is used, a very small dosage of active material may produce an inordinately large increase in the yeast crop. The crops are likely to be irregular and there is an unnecessary loss of time. These statements apply primarily to cases in which yeast is grown without agitation.

4. In spite of the uniformities above noted, each of several different strains of yeast (so-called *Saccharomyces cerevisiae*) reacts more or less distinctively toward different "bios" preparations. In some cases the contrast in behavior is very marked. This indicates possible deep-seated differences in the metabolic processes in different strains.

5. The results show that our most concentrated fraction (adsorbable by fuller's earth) in conjunction with the unadsorbed residue stimulates two of the yeasts markedly in very small doses and has a varying though definite effect on the others. In the case of yeast No. 578, doses of this concentrate as low as 0.00005 mg. per cc. have a marked effect. The same preparation has little effect on one or two of the yeasts tested and uniformly has little effect on any yeast unless the "unadsorbed residue" is also present. The dosage indicated above is, to the best of our knowledge, much smaller than that used by any other investigators in this field.

6. The effects of "α-Bios" and "β-Bios" and that of inositol on the six yeasts tested are of a lesser order and involve the presence of relatively large amounts of the substances. It seems to us within the realm of possibility that such activities as these preparations possess may be due to occluded or adsorbed impurities.

7. Miss Eastcott's conclusion as to the identity of inositol with a bios (Bios I) does not appear justified by her evidence. Until more convincing proof is offered we must tentatively regard its yeast growth stimulatory effect as probably due to impurities.[6]

EUGENE, OREGON

[6] Miss E. V. Eastcott thanks the Editor for an opportunity to read the manuscript of Williams, Warner and Roehm and to point out that, except in their own paper, the substance to which Mr. Lucas gave the name "Bios I" and which has since been identi-

fied with inactive inosite, has never been spoken of as "a bios." It is one of the constituents into which Lucas separated Wildiers' bios; by itself it has little if any effect on the yeast crop; but if the other constituent, namely, "Bios II," be present in the culture medium, addition of inosite much increases the crop. As to the inosite from tea, Miss Eastcott's paper, *J. Phys. Chem.*, **32**, 1094 (1928), states that it was recrystallized from methyl alcohol and water and obtained in two "clear white" crystalline forms, anhydrous and the dihydrate, both of which were analyzed; also that it made no difference in the yeast crops obtained with various preparations of Bios II whether Kahlbaum's inosite was employed or that from tea. Her laboratory note-books show that the last six recrystallizations had no effect on the "activity" of the latter.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, UNIVERSAL OIL PRODUCTS COMPANY]

INTERACTION OF ALKYL SULFIDES AND SALTS OF MERCURY

BY W. F. FARAGHER, J. C. MORRELL AND S. COMAY

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The property of organic sulfides of forming compounds with certain heavy metal salts was discovered by Loir.[1] He prepared compounds of methyl and ethyl sulfides with mercuric chloride, mercuric iodide and platinum chloride.

Some of the results reported by Loir were criticized by subsequent investigators. Blomstrand[2] found the properties of the product formed by the interaction of ethyl sulfide and platinic chloride to be different from those reported by Loir: the melting point was 70° higher. Abel,[3] who discovered ethyl sulfide in dog's urine, contested the originally reported melting point of the compound of ethyl sulfide and mercuric chloride.

Phillips[4] disputed the formula assigned by Loir to the reaction product of methyl sulfide and mercuric chloride. He found that the compound was $3HgCl_2 \cdot 2(CH_3)_2S$ and not $(CH_3)_2S \cdot HgCl_2$, as stated by Loir. The reason for the conflict between reports is presented in this paper.

The compounds of alkyl sulfides and salts of platinum were quite extensively investigated by Blomstrand,[2] Blomstrand and his co-workers,[5] Klason[6] and Rây.[7]

On the other hand, the literature contains little information about the compounds of alkyl sulfides and salts of mercury. Smiles[8] reported the preparation of five addition compounds of alkyl sulfides and mercuric iodide: $(CH_3)_2S \cdot HgI_2$, $(CH_3SC_2H_5) \cdot HgI_2$, $(C_2H_5)_2S \cdot HgI_2$, $(C_5H_{11})_2S \cdot HgI_2$ and $(C_6H_5CH_2)_2S \cdot HgI_2$.

[1] Loir, *Ann.*, **87**, 369 (1853).

[2] Blomstrand, *J. prakt. Chem.* [2] **27**, 190 (1883).

[3] Abel, *Z. physiol. Chem.* (Hoppe-Seyler) **20**, 269 (1895).

[4] Phillips, THIS JOURNAL, **23**, 254 (1901).

[5] Blomstrand and others, *J. prakt. Chem.*, [2] **38**, 353 (1888).

[6] Klason, *Ber.*, **28**, 1493 (1895).

[7] Rây, *Quart. J. Indian Chem. Soc.*, **2**, 178 (1925).

[8] Smiles, *J. Chem. Soc.*, **77**, 163 (1900).