TABLE IV

Effect of various metabolites added to the growth medium on the acetylation of sulfanilamide in vitro

Supplements added to growth medium	Concentration µg/ml	Acetyl sulfanilamide formed per mg dry weight of cells	Acetyl PABA* formed per mg dry weight of cell
1. None		1·698	5·25
2. *p*ABA ..	50	..	4·90
3. Sulfanilamide ..	125	1·483	..
4. 3 + Calcium pantothenate	10	1·74	..
5. 4 + *dl*-aspartic acid	25	1·98	..
6. 5 + Sodium acetate ..	50	2·36	..

* *In vitro* reaction mixture for *p*ABA contained 7 µ moles of *p*ABA instead of sulfanilamide. Acetyl *p*ABA was computed from the difference in the arylamine before and after hydrolysis.

Methionine has been shown to spare to some extent, the requirement of vitamin B_{12} in this organism. Also methionine has been shown to increase the calcium pantothenate levels in *E. coli* and *B. subtilis* grown independent of vitamin B_{12}[11].

The demonstration of *in vitro* acetylating system in *O. malhamensis* was established with respiring cells (Tables III and IV). An interesting point of the present study is the rather long period required for the maximum conversion of sulfanilamide. Probably permeability factors are involved in this. Similar results were observed in the case of *p*ABA acetylating enzyme[6] in this organism.

Aerobic conditions favoured the synthesis of acetyl sulfanilamide *in vitro* as provided by incubating the system in rotary shakers.

With pigeon liver extracts the similar results have been observed[12,13]. The present studies demonstrate the development of sulfa-acetylating system in *O. malhamensis* in presence of sub-inhibitory levels of the drug.

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ANALYSIS OF METHACROLEIN IN GAS MIXTURES

METHACROLEIN, an unsaturated aldehyde, can be estimated by several methods[1] applicable to carbonyl compounds, such as oxime method, hydrazone method, bisulphite method, dimedone method[2] and polarographic method[3]. However, these have been developed for analysis of carbonyl compounds only in solution. A method is described here for estimating methacrolein in gas mixtures.

Methacrolein is absorbed quantitatively in concentrated sulphuric acid and so can be estimated in this way. The method cannot be used, however, if the methacrolein vapour is mixed with gaseous olefins. The following method has been successfully employed for analysing methacrolein in product gas mixtures that result from air oxidation of isobutene over bismuth molybdate or ferric molybdate catalyst[4] at 350°–500° C, and consist of isobutene (I), methacrolein (II), carbon monoxide (III), carbon dioxide (IV), oxygen (V), and nitrogen (VI), a typical composition being I 5·0%, II 1·5%, III 1·0%, IV 1·0%, V 16·0% and VI 75·5%. An orsat-type gas analysis set-up is employed. For absorbing methacrolein a saturated solution of dimedone (5 : 5-dimethyl cyclohexane-1 : 3-dione) in an aqueous solution of sodium sulphate (12%) is used.

While dimedone is the active reagent forming an insoluble product with II, sodium sulphate is added only to reduce the solubility of other gases in the solution. It is however found that though the reagent does not absorb I, III and V, it does absorb IV to some extent. In view of this, analysis is done twice taking two samples of the same gas mixture. In one case, the total volume (V_1) of II and IV in the gas mixture is measured by absorption in dimedone solution followed by KOH solution, and I, V and III are then estimated, in this

sequence, by absorption in appropriate reagents. In the other case, the total volume (V_2) of I and II is first measured by absorption in syrupy phosphoric acid saturated with P_2O_5 (phosphoric acid is used since it does not absorb IV while 96% H_2SO_4 does to some extent, though both absorb I and II quantitatively) and then IV by absorption in KOH solution, followed by V and III as before. The volume of II is obtained by subtracting IV of the second analysis from V_1 of the first as also by subtracting I of the first analysis from V_2 of the second. These two values are found to agree within 4%, indicating the reliability of the method. The data of Table I show the effectiveness of the dimedone reagent, compared to concentrated H_2SO_4, in removing methacrolein from gaseous mixtures.

TABLE I

Comparison of methacrolein estimation by absorption in concentrated H_2SO_4 and dimedone solution

Percentage of methacrolein in gas mixture		% Deviation
96% H_2SO_4	Dimedone solution	
9·64	9·45	2·0
7·27	7·12	2·1
6·04	5·90	2·3
4·16	4·05	2·6
1·50	1·45	3·3

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COMPONENTS OF THE SEED COATS OF *PHASEOLUS MUNGO* AND *PHASEOLUS RADIATUS*

THOUGH a good amount of work has been done on the amino acids, proteins, enzymes and carbohydrates of *P. mungo* (green gram) and *P. radiatus* (black gram) seeds and their nutrient value, no work has so far been reported on the polyphenolic and related components. Information about them was needed for biological studies.

Miyamichi *et al.*[1-2] reported the isolation of a saponin, m.p. 202°, from the acid portion of the seed extract of *P. radiatus*; its constitution has not yet been completely established. The acid portion also contained palmitic, stearic, arachidic, linolic, linolenic and oleic acids.

For the present work, the seed coats (husk) were obtained as follows: (1) the seeds were soaked in water for 4–5 hr and then rubbed with the hand. While the kernel submerged, the seed coats floated. They were collected and dried in the shade. (2) The seed coat was separated after milling the seeds in a small stone mill. In this connection a special observation was made. Samples of these seeds had been kept in a place accessible to rats; the black gram was left untouched and the green gram was dehusked and the kernels eaten. Explanation of the behaviour of the rats is not clear. Can the presence of polyphenols in the husk be responsible? The cleanly separated seed husk was used in the study. The samples obtained by all these methods gave the same result.

The hot alcohol extract of both *P. mungo* and *P. radiatus*, husks was separated into 2 fractions using ether.

(1) Ether insoluble fraction of both on elution with methanol : chloroform (15 : 85) from a column of silica gel, yielded the same yellow crystalline substance, m.p. 265–66° (decomp). Identity of the samples was proved by TLC, paper chromatography and m.m.p.

The compound gave brown colour with alcoholic $FeCl_3$, positive Mg/HCl and negative Zn/HCl tests. Though it gave positive Molisch's test, it was not hydrolysed by Kiliani mixture or 7% sulphuric acid. Hence the compound is a flavonoid C-glycoside. The u.v. data[3] suggested the compound to be vitexin and a comparison with an authentic sample of vitexin (TLC, paper chromatography, m.m.p. and IR) confimed the identity.

The ether insoluble portion of *P. mungo* did not yield any other flavonoid; however that of *P. radiatus* contained another phenolic component eluted by methanol : chloroform (1 : 4) from the column, in very minor amounts, highly contaminated with sugars. Apart from these, this fraction of both husks contained considerable amount of sugars and therefore the husks have some nutritive value.

(2) The ether soluble portion of *P. mungo* and *P. radiatus* gave a hydrocarbon, m.p. 85–87°, by column chromatography. Another compound, m.p.