AN EXAMINATION OF SOME GUM-ENZYMES.

By Gilbert J. Fowler and M. A. Malandkar.

In the course of work on the preparation and the chemistry of gum from the gum-oleo-resin of *Boswellia Serrata*,[1] it was frequently noticed that the gum mucilage, obtained on autoclaving the gum-oleo-resin with water, became black on the surface when exposed to air. Subsequent investigation showed that this was occasionally due to fungoid growth, but such an explanation was not completely satisfactory in all cases, as, e.g., when the gum in a moist condition, after extraction of the resin with alcohol, darkened on the surface in a few hours. This phenomenon recalled the behaviour of Japanese gum-lac, and pointed to the presence of an oxidase. On testing the alcohol-extracted gum with guaiacum tincture a strong blue reaction was obtained.

As no reference could be found to the occurrence of enzymes in *Boswellia*, the subject appeared worthy of further inquiry, and it was decided carefully to study all the enzymes whose presence could be detected in *Boswellia* gum, and compare them with other gums from gum-oleo-resins, e.g., myrrh, and in true gums such as gum-arabic. It was also decided to attempt to determine whether such enzymes play any part in the natural processes concerned with gum formation.

An excellent summary of the literature on gums is given by Reinitzer [2] and has been used in cases of reference to which access was not possible. From this it will be seen that the subject of gum-enzymes presented several unsolved problems. It appeared to be established that certain acacia-gums contain oxidising and diastatic enzymes. Whether the latter were identical with malt diastase was not determined. There were no comparative data with regard to the enzymic activity of gums from gum-oleo-resins and of true gums. The nature of the oxidising enzymes present had not been studied in the light of recent theories of the oxidation processes occurring in presence of such enzymes. Moreover, the important question of the relation between enzymes present in gums and the process of gum-formation had not been systematically studied.

[1] *This Journal*, 1921, 4, 27.
[2] *Zeit. f. physiol. Chem.*, 1909, 61, 352.

The investigation described in the present paper is an attempt to throw light on some of the foregoing questions. The following gums served as material for study :—

1. *Boswellia serrata.*
2. Myrrh.
3. Gum-arabic, varieties A and B.
4. Gum from *Cochlospermum gossypium.*
5. Gum-kino from *Butea frondosa.*
6. Gum from *Moringa pterigospermum.*
7. Two gums sent from Dehra Dun described as *Sterculia.*

Of these gums *Boswellia* and myrrh represent gums from gum-oleo-resins. The two varieties of gum-arabic represent typical acacia-gums.

No. 4 is characterised by constantly giving off acetic acid, recognised by smell on opening a bottle containing the gum. This gum also absorbs large quantities of water with consequent swelling. Gums with these properties are obtained from *Cochlospermum gossypium* and allied species,[1] so that the particular gum under examination is given this general distinctive name. The gums *Butea* and *Moringa* are characterised by a large tannin-content, indicated by the production of a blue-black coloration with ferric chloride. *Sterculia* resembles *Cochlospermum gossypium* in swelling with absorption of water, but it does not evolve acetic acid. It appears that some *Sterculia* gums yield acetic acid, but the specimens in our possession did not.[2]

No enzymes, or only traces could be found in any of the gums available except the first three in the above list. These, however, contained both oxidising and diastatic enzymes, the first two exhibiting much greater activity than the third. None of the gums contained tyrosinase, invertase, or maltase. These oxidising and diastatic enzymes have been carefully examined. The manganese and nitrogen-contents of the gums have been determined in view of their possible relation to enzymic changes.

The gums have also been examined for other enzymes especially those acting upon tannins, as such enzymes may play some part in the process of gum-formation in nature.

[1] Robinson, *J. Chem. Soc.*, 1906, 89, 1496.
[2] Guilourt, *Pharm. Jour.*, 1895, 15, 57.

EXPERIMENTAL.

Preparation of Gums.

Boswellia and myrrh gums were freed from their accompanying oleo-resin by systematic exhaustion with alcohol. The oleo-resin was shaken with 90 per cent. alcohol and the dissolved oleo-resin decanted. This procedure was repeated half a dozen times, when practically all the oleo-resin had passed into solution. The lumps were then powdered with alcohol in a mortar and the powder washed into a bottle with alcohol. The powder was washed four times by decantation with alcohol, transferred to a Buchner funnel, washed twice with alcohol and twice with ether. It was then dried over sulphuric acid for two days *in vacuo* and one day in air, and bottled for use. The gum prepared in this way is termed 'cold-extracted gum.' Some tests were carried out on the *Boswellia* gum prepared by extracting the gum-oleo-resin in a Soxhlet apparatus. This material is called 'hot-extracted gum.'

The gum-arabic was divided into two parts according to colour. The white pieces which did not produce a blue colour with guaiacum tincture without hydrogen peroxide were termed gum-arabic (B), while brownish lumps which gave a slight blue colour with guaiacum alone were termed gum-arabic (A).

Oxidising Enzymes.

Qualitative tests.—In the case of cold-extracted *Boswellia* and Myrrh gums the maximum intensity of blue colour with guaiacum was attained after about half-a-minute, gum-arabic (A) requiring about a minute, when the colour was less intense than that produced by the other two.

This power of giving colour with guaiacum was inhibited by two drops of concentrated sulphuric acid or by boiling the gum solution for five minutes; after five minutes boiling the colour did not appear again with guaiacum either alone, or with hydrogen peroxide. Tannic acid had the same effect but rather a large amount was found necessary for complete inhibition. The colour with guaiacum alone was produced by the *Boswellia* gum, though with diminished rapidity and intensity even after it had been heated in a dry state in a steam-oven for eighteen hours. *Boswellia* and myrrh gums when freshly prepared gave a very faint peroxide reaction with potassium iodide, starch and acetic acid, but curiously enough no peroxide reaction was shown when sulphuric acid replaced acetic. Under the same conditions,

organic peroxides such as oxidised turpentine oil and fresh potato-juice were found to give the peroxide reaction when sulphuric acid was used rather more readily than with acetic. No peroxide reaction took place with gum-arabic. A few days after their preparation, however, the peroxide reaction was no longer given by *Boswellia* and myrrh gums; hydrogen peroxide could not be detected in these, though several tests were employed, including titanium sulphate and the method described by Bach.

It may be stated in this connection that Bach and Chodat showed that the juice of *Lathraea squamaria* contained a substance which turned potassium iodide-starch-paper blue, but did not react with titanium sulphate. Nitrous acid was shown to be absent and therefore the substance was taken to be a peroxide. In the present case the substance may be similar.

It has been pointed out by Moore and Whitley [1] that potato-juice exposed to air in a shallow dish for about an hour loses its power of blueing guaiacum without hydrogen peroxide. This was confirmed. Gum solutions do not behave in the same way, i.e., they give with guaiacum a blue colour practically as intense as when they are fresh.

It is generally supposed that the oxidase, or the substance giving a direct guaiacum reaction, is destroyed by keeping the solution at 70° for 10 minutes. This was not so with *Boswellia* gum-mucilage which only completely lost its power of producing colour, with or without hydrogen peroxide, after some eight hours at 70°.

The guaiacum test being a very general one, given by blood and by other substances exercising a somewhat indefinite oxidising action, some additional, more specific reagents were sought in the present case. Of the various oxidisable phenols and aminophenols available, three were employed, viz., *a*-naphthol, *p*-phenylenediamine and Spitzer's reagent. The details with regard to the preparation of guaiacum tincture and of these reagents, and some observations on their reactions are given in the following paragraphs :—

1. Two per cent. of guaiacum resin is dissolved in 85 per cent. alcohol. This tincture was found to give an exceedingly faint reaction for peroxides. To destroy these traces of peroxides, the tincture was boiled for ten minutes under a reflux condenser with a little animal charcoal. The filtered tincture was used for the test,

[1] *Biochem. J.*, 1909, 4, 136.

and did not give any peroxide reaction for three days, when it began to show the presence of peroxides.

2. One per cent. of α-naphthol is dissolved in fifty per cent. alcohol. It strikes a fine amethyst colour in presence of an oxidase.

3. One per cent. of *p*-phenylenediamine is dissolved in distilled water. With *Boswellia* gum it did not give the fine green colour described by Moore and Whitley, but a colour which appears greenish black by reflected light and dark violet by transmitted light. It gave a similar colour with potato-juice.

4. Spitzer's reagent, or indophenol was prepared fresh in all cases by mixing equal parts of the two previous solutions of α-napthol and *p*-phenylenediamine and a two per cent. solution of sodium carbonate. This gave a fine purple colour. A very slight development of colour occurs in boiled gum solutions used as controls, but the colour was insignificant compared with the colour in unboiled gum solutions.

In making these comparative tests care was taken to use similar gum solutions. The solutions contained 2·5 gms. of dry gum in 100 c.c. of water. The control solutions were previously boiled for five minutes. The proportion of reagent to gum solution was 0·5 c.c. to 4 c.c. Where hydrogen peroxide was added two drops were used. The results are tabulated below :—

'Cold-extracted' *Boswellia* and myrrh gums.

Reagent	Colour with gum solution	Colour with gum solution H_2O_2
Guaiacum	Blue	Blue colour hastened.
α-Naphthol	Amethyst	Amethyst hastened.
p-Phenylenediamine ...	Greenish black	Deep greenish black.
Indophenol	Purple	Deep purple.

In the case of gum-arabic (A) the colours with the gum solution alone were all somewhat faint, but were intensified by hydrogen peroxide. Gum-arabic (B) gave no colour with the first three reagents and only a slight purple with indophenol, but hydrogen peroxide developed distinct colours. Except the faint purple already mentioned in the case of indophenol, none of the other reagents gave any colour with the controls.

It is evident from the above results that the oxidase action in the case of *Boswellia* and myrrh gums is much stronger than in the case of gum-arabic. Acceleration of colour-development by hydrogen peroxide also shows that the gums contain a peroxidase.

Quantitative methods for measuring the oxidase activity.

An attempt was made to measure quantitatively the differences in oxidase activity of the gums. Two methods were tried, one volumetric and the other gravimetric.

1. *Manometric method.*—A modification of Bunzel's[1] method was employed, according to which the amount of oxygen absorbed is measured by the fall in pressure indicated by a manometer. A known amount of enzyme solution is allowed to act on a known amount of pyrogallol in presence of air. The oxygen is transferred from air to pyrogallol by the enzyme. The equivalent quantity of evolved carbon dioxide is absorbed by a suitable reagent and the diminution in pressure noted.

The experiments were carried out in a Schrötter's apparatus of about 75 c.c. capacity instead of in a Bunzel's apparatus. 8 cc. of a freshly prepared 1 per cent. pyrogallol solution in saturated thymol water, together with 4 c.c. of a 1 per cent. solution of the respective gum were placed in the bulb, the thymol inhibiting bacterial action. The enzyme solution was placed in the tap-tube. Soda-lime was placed in the escape-tube to absorb carbon dioxide. To this end of the apparatus a manometer was attached. The whole apparatus with the two liquids was kept in a constant temperature incubator regulated at 35° for about 45 minutes, after which the incubator was slightly opened, the enzyme-solution admitted and the incubator closed. After twenty minutes the manometer was read through the glass door of the incubator, when the door was slightly opened, the apparatus taken out and gently shaken for about half-a-minute, the door of the incubator being shut immediately after removing the apparatus which was quickly replaced. The shaking was repeated every twenty minutes for the first five hours and then after twenty hours every hour for six hours. As vigorous shaking is known to cause some enzymes to become less active, the apparatus was shaken gently and uniformly in all cases. Before reading the manometer the apparatus was allowed to remain in the closed incubator for twenty minutes. The temperature of the incubator and the barometric pressure were read at the same time.

[1] *Jour. Amer. Chem. Soc.*, 1912, 34, 303.

The maximum temperature variation of the incubator was found to be 0·3°. As the object of these experiments was to get comparative results, the correction for the barometric pressure only is applied in the following figures, the slight correction due to variation in temperature being neglected.

Preliminary experiments showed that the action was slow. The following figures give a measure in millimetres of the fall in pressure at the end of 4, 20 and 44 hours. Potato-juice was examined for comparison with the values obtained by Bunzel :—

Substance	Fall of pressure in			Ratio of substance to water
	4 hours	20 hours	44 hours	
Potato-juice	5	25	38	20 grams to 20 grams.
Potato-juice boiled	1	2·5	5·5	Do.
Cold-extracted *Boswellia* gum ...	14	69	131·5	10 grams to 100 c. c.
Boswellia boiled	2	2·4	4	Do.
Cold-extracted myrrh gum ...	14	68	125	Do.
Myrrh boiled	1	2	4	Do.
Gum-arabic (A)	2·0	21	37	10 grams to 100 c. c.
Gum-arabic boiled	1·0	3	5·5	Do.
Hot-extracted *Boswellia* gum ...	11·0	40	71	Do.
Boswellia gum heated for 18 hours in the steam oven	9·0	33	56	Do.
Gum prepared by the cooking method	0·5	3	...	Do.

It is interesting here to note the variations in results from those obtained by Bunzel in the case of potato-juice. Bunzel, employing sodium hydroxide to absorb carbon dioxide, showed that the reaction came to an end in about two hours. Edal Behram's [1] results with the oxidase in the leaf of *Lantana camara* show that the reaction was slow and came to an end after about forty hours. Edal Behram's experiments were carried out in a similar manner to our own, except that he employed a shaking arrangement in the incubator.

It is evident from the results that *Boswellia* and myrrh gums exhibit much stronger oxidase activity than gum-arabic. It is also clear that the gum prepared by the autoclave process is inactive, and

[1] *This Journal*, 1919, 2, 195.

as this gum contains a little resin, the activity in the 'extracted' gum cannot be simply due to the presence of resin. Further the results show that the oxidase activity is destroyed practically completely by boiling the gum solutions for five minutes, and that heating the solid gum in the steam-oven for eighteen hours only diminishes the oxidase activity, but does not altogether destroy it.

2. *Gravimetric method.*—Known amounts of gums were allowed to act on pyrogallol for a definite time and the purpurogallin weighed. Identity with purpurogallin was proved by solubility in water, crystalline appearance, melting point and behaviour towards sulphuric acid and sodium hydroxide. It formed red needles practically insoluble in water. Its melting point was 218°, that of purpurogallin given in Beilstein being 220°. Solutions in sulphuric acid and in sodium hydroxide were red and blue respectively.

As preliminary experiments showed that the amount of purpurogallin varied with temperature and with the surface exposed to air, the experiments were carried out in an incubator at 36° and in 60 c.c. Erlenmeyer flasks adjusted to expose the same surface to air in each case. 20 c.c. of a solution of pyrogallol in saturated thymol water (20 c.c. of the solution contained the required amount of pyrogallol) were put in each flask; saturated thymol water was added so that the final volume after the addition of the gum solutions was 35 c.c. in each case. It was necessary to use weak solutions as strong solutions of *Boswellia* are impracticable to filter. After adding the gum solution, the liquid was shaken a little, lightly plugged with cotton-wool and kept in the incubator at 36° for twenty-four hours, when the liquid was filtered through asbestos in Gooch crucibles; the purpurogallin was washed with 30 c.c. of water and dried at 105° to constant weight.

The experiments were tried with cold-extracted *Boswellia* and myrrh gums and gum-arabic (A). In the case of the last named, practically no purpurogallin was found to have been produced after twenty-four hours; the amount produced in seventy-two hours was therefore determined. In the case of *Boswellia* gum the variation in the amount of purpurogallin formed with different quantities of pyrogallol was also studied. Experiments with hydrogen peroxide showed that it had an inhibiting effect on the production of purpurogallin by the *Boswellia* gum and also hindered the reaction with guaiacum, differing in this respect from its behaviour in presence of potato-juice. Whether this difference is due to a greater sensitiveness to acid on the part of the oxidase of *Boswellia* is not certain. The hydrogen peroxide used had a very slight acid reaction.

The following table shows in milligrams the amount of purpurogallin formed when differrent amounts of the gums are allowed to act on one gram of pyrogallol :—

Name of Gum	Quantities of gum used, in decigrams					
	1	2	3	4	5	6
Cold-extracted *Boswellia* gum ...	15	25	35	47	60	67
Cold-extracted Myrrh gum ...	2	3	7	13	17	20
Gum-arabic (A) after 72 hours ...	7	7	8	9	10	10

Purpurogallin was not produced with boiled gum solutions. It is evident that the oxidase activity of *Boswellia* gum is greater than that of gum-arabic. It is curious that myrrh gum, which closely resembles *Boswellia* gum in all other properties examined, produces proportionately much less purpurogallin.

The following table shows the quantity of purpurogallin formed with varying amounts of pyrogallol by 0·05 gm. of cold-extracted *Boswellia* gum :—

Pyrogallol	*Purpurogallin formed*
0·5 gm.	36·4 mgms.
1·0 ,,	60 ,,
1·5 ,,	67 ,,
2·0 ,,	78 ,,

It will be seen that the amount of purpurogallin increases with the amount of pyrogallol.

The following figures obtained when using 0·05 grm. of *Boswellia* gum and 1 gram of pyrogallol show that the formation of purpurogallin is not complete in twenty-four hours :—

Purpurogallin formed after 24 hours 60 mgms.
Purpurogallin formed after 48 do. 68 ,,

Oxidising Enzymes in Gums in Relation to Recent Theories.

The general conception of an oxidase was formerly that of an enzyme giving a blue colour directly on addition of guaiacum ; if the colour is only given on subsequent addition of hydrogen peroxide, the enzyme was referred to as a peroxidase or indirect oxidising enzyme.

The view that an oxidase consists of a peroxidase and a naturally occurring peroxide was put forward by Kastle and Loevenhart [1] in

[1] *Amer. Chem. Jour.* 1901, 26, 539.

1901. The peroxidase acts upon the peroxide and transfers oxygen in an active state to readily oxidisable substances such as guaiacum. In the case of plant-tissues which give the direct reaction it has been suggested that some organic substance in the plant acts as a peroxide, while in tissues giving the indirect reaction peroxidase only is present and the peroxide may be supplied artificially in the form of hydrogen peroxide.

The conception due to Chodat and Bach [1] is rather more complex than the one outlined above. The authors regarded an oxidase as consisting of two components, a peroxidase (as described above) and an oxygenase. Chodat speaks of oxygenase thus: 'es sind fermentartige körper die sich mit dem Sauerstoff der Luft zu einem Peroxyd verbinden können.' Thus they do not differentiate clearly between the organic compounds from which the peroxide is formed and the enzyme which forms the peroxide.

Moore and Whitley from numerous experiments conclude that the sole difference between the various plant extracts, etc., which show an oxidising action consists in the presence of a small variable amount of chemically unstable peroxide.

Onslow [2] in 1919 showed that the tissues and tissue-extracts of plants which turn brown on injury and give a direct oxidase reaction with guaiacum tincture, contain some substance giving reactions characteristic of the catechol grouping in addition to a peroxidase; in the case of other plants which do not turn brown on injury the blueing of guaiacum occurs only after addition of hydrogen peroxide, and from these plants substances with the catechol grouping are absent. In the first type of plants the tissues could be freed from the catechol substance by thoroughly pounding and extracting with cold alcohol. The water-extract of the tissue-residue will then turn guaiacum blue only on addition of hydrogen peroxide. When this extract is added to solutions of substances containing the catechol grouping, or the compound from the plant itself, oxidation of these also takes place. The resultant combination will then turn guaiacum blue. Enzyme extracts prepared in a similar way from plants devoid of catechol substances are without effect on guaiacum, etc. This led Onslow to suggest the presence of a second enzyme termed oxygenase in addition to peroxidase in the plants giving the direct reaction. The function of the former being to catalyse the auto-oxidation of the catechol compound with the formation of peroxides; the latter are then acted upon by peroxidases and produce active oxygen. The complete system, i.e. oxygenase, catechol-like substance and peroxidase constitute an oxidase.

[1] *Ber.*, 1903, 36, 606. [2] *Biochem. J.*, 1919, 13, 1.

An attempt was made to study the gums under examination in the light of this theory. The gum was pounded and washed with alcohol in the cold to remove the 'catechol substance.' In the case of *Boswellia* and myrrh gums, though the intensity and rapidity of colour-production with guaiacum tincture alone diminished with repeated washings, still even after 50 washings the gum very slowly gave a blue colour with guaiacum tincture. This may be due to some intimate union of the 'catechol substance' with the gum.

Onslow's method for extracting the 'catechol substance' was to drop the slices of tissue in boiling 96 per cent. alcohol, filtering the hot alcohol after boiling for some time, removing the alcohol by evaporation *in vacuo*, precipitating the remaining liquid with concentrated lead acetate solution, decomposing the pale yellow lead precipitate with sulphuric acid, then filtering and almost neutralising the filtrate with potassium hydroxide. Though a slight precipitate could be obtained by this method in the case of *Boswellia* and myrrh gums, it was not pale yellow and no 'catechol' reaction could be obtained with the final liquid. After some trials the following modification was found to be successful.

The gum-oleo-resin was freed from the oleo-resin by shaking with alcohol; the lumps were slightly crushed and kept in 70 per cent. alcohol for a week. The alcohol was then filtered and when a drop of dilute ferric chloride was added to the filtrate a transient greenish colour was produced. When immediately after the production of the greenish colour, two drops of ammonia were added to the liquid a buff coloured precipitate was produced quite distinct from that of ferric hydroxide. Alcohol kept in a similar way without the gum showed no such colour, nor was the colour due to resin, as a dilute alcoholic solution of resin gives with dilute ferric chloride a deep brown colour.

When the gum solution was added to an aqueous solution of pure catechol, a yellowish tinge was found to have rapidly developed, eventually deepening to a yellowish brown in about an hour just in the same way as the peroxidase, in the investigations of Onslow. When tincture of guaiacum was added to this, an intense blue colour was produced at once, and therefore much more rapidly than was the case when the gum alone was added.

It is needless to say that a dilute solution of pure catechol alone did not give any of the above colorations or reactions with guaiacum under similar conditions. No catechol-like substance was detected in the alcoholic extract from gum-arabic, the colour always being dark brown.

Although too much reliance should not be placed on such colour reactions, which are apt to vary with concentration and other conditions, yet the general evidence does point to the presence of a very small quantity of a substance giving the reaction of catechol-grouping in the extracted gum of *Boswellia* and myrrh, and that the oxidase system in the case of these gums consists of a catechol-like substance, oxygenase and a peroxidase.

Tyrosinase.—On adding 4 c.c. of 5 per cent. gum solution to 10 c.c. of a cold saturated aqueous solution of tyrosine, practically no change of colour was observed after 24 hours. This confirms the general opinion that tyrosinase is not present in gums.

Detection and Estimation of Manganese.

In the case of laccase, Bertrand found that the activity of the ferment is directly proportional to the amount of manganese present. Reinitzer, on the other hand, found in the acacia-gums he examined that manganese was either absent or occurred in traces only. We therefore examined our three selected gums with special reference to this question, and all showed the presence of manganese when tested with lead peroxide and nitric acid.

For the quantitative estimation of manganese the method of Berkeley [1] was employed. Briefly the method consists in careful incineration of the gum, elimination of chlorides from the resulting ash by heating with a few drops of sulphuric acid, the dry residue after sulphuric acid treatment being heated to bright redness with a trace of potassium nitrate. The cooled product is then heated first with nitric acid and hydrogen peroxide, and finally with ammonium persulphate; the permanganic acid thus formed is titrated with sodium arsenite. The results from this treatment of the three gums are tabulated below:—

Material	Percentage of managnese in the dry gum	Percentage of manganese in the ash	Percentage of ash in the dry gum
Cold-extracted *Boswellia* gum ...	0·0095	0·185	5·2
Cold-extracted myrrh gum	0·0115	0·165	7·0
Gum-arabic (A)	0·0110	0·490	2·2

[1] *Biochem. J.*, 1922, 16, 70.

The results show that manganese occurs in traces only, and there is no substantial difference in the manganese-content of the gums examined.

Diastatic Enzymes.

The three gums, *Boswellia*, myrrh and gum-arabic (A) were examined in detail in relation to the behaviour of the diastatic enzymes, whose presence had been revealed by preliminary examination. All experiments were carried out in concentrated thymol water, shown to be without action on the enzyme, contrary to the suggestion of Grafe. The starch solution was prepared from Lintner's soluble starch.

The action of gum diastase on starch was tested in the usual way by adding from time to time a drop of dilute iodine solution, and the change effected was found to be progressive as in the case of malt-diastase.

As the diastatic power, especially in the case of gum-arabic, was small, Ling's modification of Lintner's method was found the most convenient for its determination, and was carried out as follows:— 4 c.c. of a 5 per cent. gum solution were added to 100 c.c. of a 2 per cent. solution of starch in a 200 c.c. flask and kept at 30° for 1 hour (Ling's method requires 21), when 20 c.c. of N/10 sodium hydrate were added and the liquid made up to 200 c.c. with water. After mixing, this solution was gradually run from a burette into 5 c.c. of Fehling's solution diluted with a little water and kept boiling until the solution just lost its blue colour. The diastatic power was calculated according to the formula $\frac{1000}{XY}$ where X represents the number of c.c. of liquid reducing 5 c.c. of Fehling's solution and Y the c.c. of gum solution added.

In the case of gum-arabic the diastatic power must be very low since 1 c.c. of Fehling's solution required more than 50 c.c. of the liquid. The correction due to blanks with boiled gum solution was practically negligible. The results are tabulated below:—

Gum		*Diastatic power*
Cold-extracted *Boswellia* gum	....	3·7
Cold-extracted myrrh gum	....	3·6
Gum-arabic (A)	....	less than 1

Thus the diastatic power of *Boswellia* and myrrh gums is nearly the same, but is much greater than that of gum-arabic.

The sugar formed by the gum diastase was identified as follows :— One gram of the gum (in the case of gum-arabic 2 grms.) acted on 250 c.c. of a 3 per cent. starch solution at 50° for 96 hours in the case of *Boswellia* and myrrh gums and 120 hours in the case of gum-arabic. Strong alcohol was then added little by little to the liquid until a considerable amount of precipitate was formed and filtered through a thin layer of kieselguhr; the filtrate was distilled under reduced pressure to a small bulk. Alcohol was again added till no more precipitate was formed, the liquid again filtered and the alcohol again distilled. Almost all the water was evaporated on a water-bath, the residue was then dried *in vacuo* over sulphuric acid, and the osazone prepared from the residue in the usual way. Its solubility in hot water and its crystalline appearance under the microscope pointed quite clearly to the exclusive presence of maltose. A test for glucose by the special method described by Ling and Nanji [1] was negative.

Estimation of Sugar formed by the Gum-diastase.

Careful determinations were made of the amount of sugar produced when equal amounts of gums acted on equal volumes of starch solutions containing the same amount of starch under similar conditions. In these experiments the sugar was estimated by Bertrand's method which in principle consists in boiling a known quantity of sugar solution with excess of a standard solution of copper hydrate. The precipitated cuprous oxide is then, according to the method devised by Mohr, dissolved in an acid solution of ferric sulphate, with formation of cupric sulphate and ferrous sulphate, the latter being estimated by titration with standard permanganate.

The experiments were conducted in the following manner:— A solution of starch was prepared in such a way that 80 c.c. of it contained one gram of starch. 80 c.c. of this solution was put into a 200 c.c. measuring flask and 100 c.c. thymol water added. One gram of gum dissolved in 10 c.c. of thymol water in a mortar was added to the liquid. The gum adhering to the mortar was immediately washed with 5 c.c. of thymol water into the flask. The volume was then made up to 200 c.c. and the liquid after mixing was poured into a 300 c.c. Erlenmeyer flask lightly plugged with cotton-wool and the flask was kept at 45°. The sugar in 10 c.c. of this liquid was estimated after 5, 22 and 28 hours.

[1] *Biochem. J.*, 1923, 17, 593.

The results given in the table below represent milligrams of sugar found in 10 c.c. of the reaction mixture calculated as maltose, after applying the slight correction due to reduction in the blanks:—

Gum	Sugar found after		
	5 hours	22 hours	28 hours
Cold-extracted *Boswellia* gum ...	25	26·5	27
Cold-extracted myrrh gum	24·5	26	27
Gum-arabic (A)	less than 10	10	13

The results show that *Boswellia* and myrrh gums produce sugar from starch solution much more readily than gum-arabic.

The Extent of Starch Conversion by Gum-diastase.

The extent to which starch is converted by cold-extracted *Boswellia* and myrrh gums can be seen from the following table where the figures given above are represented as grams of maltose from 100 grams of starch, which is called the percentage conversion of starch.

For comparison, 1 c.c. of an extract of diastase prepared from jawar was added to 80 c.c. of the starch solution, the volume made up to 200 c.c., the liquid kept at 45° after pouring it into a 300 c.c. conical flask and the sugar determined after 5, 22, and 28 hours. In the figures given below the correction due to reduction in the blank with boiled diastase extract is applied:—

Material	Percentage conversion after		
	5 hours	22 hours	28 hours
Cold-extracted *Boswellia* gum ...	50·5	53·0	54·0
Cold-extracted myrrh gum	49·0	52·0	53·5
Malt	52·0	79·5	80·0

The results show that the conversion of starch by *Boswellia* and myrrh gum-diastase becomes exceedingly slow when the copper reduction corresponds to about 50 grams of maltose. For all practical purposes it can be said that a state of equilibrium is reached at that point.

Special examination showed that gum-diastase had no action on undissolved starch granules, due probably to the absence of cellulose-dissolving enzymes.

Whilst according to Reinitzer, there are two diastatic enzymes in gums, one of which only is destroyed by treatment with 0·5 per cent. solutions of mercuric chloride, we have found that this solution completely inhibits diastatic activity.

Cellulose and hemi-cellulose-dissolving Enzymes.

Careful chemical and microscopical observations using thin sections of potato, banana-stem and a small piece of newspaper, failed to reveal any cytolytic action in a 10 per cent. solution of each of the three gums. Freshly exuded gum-arabic from a tree in the Institute compound also exhibited no tendency to dissolve hemi-cellulose.

Detection and Estimation of Nitrogen.

It has already been mentioned that Tschirch could not detect nitrogen in gums by the process of Lassaigne. Reinitzer stated that nitrogen could be recognised in acacia-gums with difficulty by the use of potassium instead of sodium. It was therefore thought neccessary to investigate the point and also to find out any difference in the gums as regards the nitrogen-content.

(*a*) *Detection of Nitrogen.*—Nitrogen can be detected in *Boswellia* and myrrh gums by the method of Will and Varrentrap, namely, heating the gum in a hard glass test tube with soda-lime, when ammonia could be observed by smell as well as by litmus. In the case of gum-arabic the fumes are very acidic and mask the comparatively small amount of ammonia; this, however, can be detected by filter-paper moistened with Nessler's solution.

Nitrogen can also be detected by Lassaigne's method; an intense blue was obtained with *Boswellia* and myrrh gums, but only a faint tint was given by gum-arabic.

(*b*) *Estimation of Nitrogen.*—The total nitrogen was estimated by Kjeldahl's method. The results are tabulated below:—

Substance	Percentage of nitrogen on weight of dry material
Cold-extracted *Boswellia* gum	3·03
Cold-extracted myrrh gum	3·02
Gum-arabic	0·16

Thus nitrogen can be detected both by the method of Lassaigne and Will and Varrentrap. It is also clear that *Boswellia* gum and myrrh gum contain practically the same amount of nitrogen which is greater than that contained in gum-arabic.

Protein Reactions.

All three gums gave a positive xanthoproteic reaction. The biuret reaction was positive with *Boswellia* and myrrh gums and negative with gum-arabic. Millon's reagent gave with *Boswellia* and myrrh gums a white precipitate which did not become pink, but remained white and was soluble in excess of the reagent. Gum-arabic gave no precipitate. It would appear, therefore, that the combination-state of the nitrogen is similar in *Boswellia* and myrrh gums, but is different in gum-arabic.

Role of Gum-enzymes in the Formation of Gums.

In view of the reversibility of enzyme action which has been proved in numerous cases, it appeared possible that gums might originate from the condensation by enzyme action of some simpler substances, such as a sugar, on a colloidal surface. In such a case the gum-enzymes acting upon a dilute gum solution might be expected to break down the complex gum-molecule into simpler substances like sugar. The presence of an excess of tannin in gum-producing plants such as acacia suggested the possibility that tannin might be in some way connected with the formation of gums. An attempt was therefore made to investigate these two points.

No evidence of any decomposition of the gums was obtained when 2·5 per cent. solutions were kept unheated for three weeks, as compared with corresponding blank solutions which had been boiled to destroy the enzymes.

Evidence, however, was obtained that action of some kind was exerted by the gum-enzymes on gallotannic acid. The reaction was carried out in a 250 c.c. Erlenmeyer flask. 100 c.c. of a 0·12 per cent. solution of gallotannic acid were taken, and 15 c.c. of a 10 per cent. solution of the gum were added to it. The flask was loosely plugged with cotton-wool, after adding a small piece of thymol and 8 c.c. of toluene as antiseptics. The flask was kept in a dark chamber at room-temperature. The amount of gallotannic acid present in the reaction mixture was estimated by the Loewenthal-Proctor method, immediately after the addition of the gum solution, and again after 24 and 120 hours. The colour of the reaction mixture

began to turn brown about fifteen minutes after the addition of the gum solution, while in the case of the blank with boiled gum solution the colour did not change appreciably even after 120 hours. A quantitative examination showed, however, that a very small amount of gallotannic acid had in fact disappeared, presumably by auto-oxidation. A correction was therefore applied to the figures obtained from the unboiled solutions, when the results were as follows :—

	After 24 hours	*After 120 hours*
Percentage reduction of tannin.	36	49

A similar experiment with a dilute, cold water extract of the wood from the region from which the gum was observed to exude in one of the acacia trees in the Institute compound, gave the following figures :—

	After 24 hours	*After 10 days*
Percentage reduction of tannin.	18	45

From the above results it will be seen that the gums as well as the wood in the vicinity of the gum contain an enzyme which acts on tannins. The nature of the product so obtained is not yet definitely determined.

CONCLUSIONS.

1. The gums examined, viz., *Boswellia*, Myrrh and Arabic contain oxidising and diastatic enzymes, those in the first two being much stronger than those in the third. This points to the fact that the enzymes in gums from gum-oleo-resins are stronger than those occurring in true gums.

2. There is evidence to show that the oxidase system in the case of *Boswellia* and myrrh gums consists, in common with other direct oxidase-systems, of a peroxidase, an oxygenase and a substance giving reactions characteristic of the catechol-grouping.

3. Manganese occurs in traces only and there is no substantial difference in its amount among the gums investigated.

4. The diastatic enzymes saccharify starch solutions, the saccharification being exceedingly slow when the amount of maltose produced amounts to about 50 per cent. of the weight of starch.

5. The enzymes do not saccharify unchanged starch or gum, nor have they any dissolving action on hemi-celluloses. Even in fresh gums hemi-cellulose-dissolving enzymes are absent.

6. The nitrogen-content of *Boswellia* and myrrh gums is much higher than that of gum-arabic. The nitrogen can be detected by the methods of Lassaigne and of Will and Varrentrap.

7. The enzymes convert tannins into non-tannins. The conversion may be an intermediate stage in the formation of gums.

Department of Bio-Chemistry,
Indian Institute of Science,
Bangalore.

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