

analogy with the behaviour of the 2:3-dihydroxytransdecalin of form B, we should expect the sapogenins to isomerise to the *trans* form on treatment with acid if these hydroxyl groups possessed the unsymmetrical configuration. Since this has not been observed, it may be concluded that in gitogenin and also in digitogenin the hydroxyl groups at C₃ and C₂ (which are in *cis* positions to each other) are *cis* and *trans* respectively with respect to the C₁₀ methyl group.

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¹ Ganapathi, *Curr. Sci.*, 1939, 3, 360.

² Noller, *J. Amer. Chem. Soc.*, 1939, 61, 2717.

³ Marker and Rohrman, *Ibid.*, 1939, 61, 2724.

⁴ *Ber.*, 1935, 68, 2248.

⁵ Ganapathi, *Ber.*, 1939, 72, 1381.

⁶ Ref. 1 footnote.

Elasticity of Organo-Gels in Relation to Hysteresis in Sorption

EXPERIMENTS on hysteresis in the sorption of vapours on organic natural colloids are few in the literature. Rao, B. S.,⁶ and co-workers have expressed the view that rice is essentially a colloidal system having the characteristics of a gel. This view can be extended to all other grains and plant materials. The unique colloidal behaviour of the rice grain⁷ of losing the hysteresis loop initially exhibited by it, when the cereal is subjected to successive sorptions and desorptions of water vapour has already been presented. This behaviour has revealed the rôle of elasticity of the swollen grain on hysteresis in sorption. This principle receives further striking confirmation by the results obtained by conducting a series of sorptions and desorptions of water vapour, on the calcium salt of gum arabic, presented in this paper.

Calcium arabate was prepared according to the method described by Briggs.² Gum arabic

(5% solution of Merck's C.P. Quality) was precipitated by ethyl alcohol from an acid solution containing hydrochloric acid (0.1 N.). It was reprecipitated and partially dried in vacuum to remove the alcohol. In order to remove the electrolytes, an aqueous solution of this gum was subjected to hot dialysis, till the dialysate showed no change in conductivity. The method developed by Bernhart¹ and co-workers was adapted for hot dialysis. The dialysed solution was just neutralised with the requisite amount of calcium hydroxide. The solution was evaporated on a water-bath till thin flakes of calcium arabate were obtained. The flakes were powdered and activated at 60° C. in vacuum for half an hour. The activated calcium arabate was degassed in the sorption tube for five hours in vacuum and a series of sorptions and desorptions of water vapour at 30° C. were conducted with the aid of a McBain-Bakr quartz fibre spring balance.⁵ The results are shown in Fig. 1.

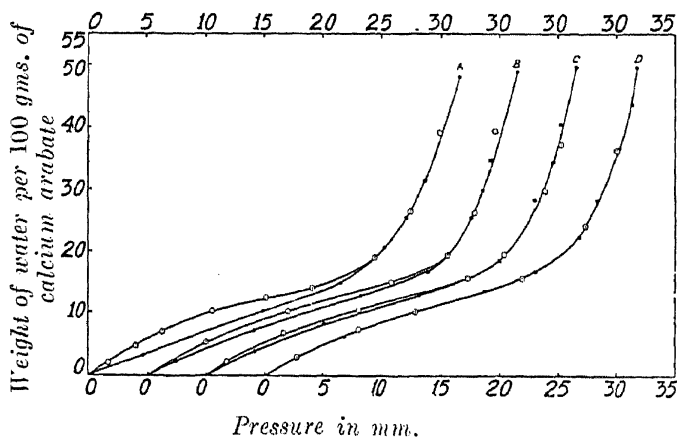


FIG. 1

- A First sorption —●—● and desorption ○—○—○
 B Second ,, ,, ,, ,,
 C Third ,, ,, ,, ,,
 D Fourth ,, ,, ,, ,,

A period of about a fortnight was necessary for completing each cycle of sorption and desorption. In the first cycle of sorption and desorption, calcium arabate exhibits a hysteresis loop which dwindles away in the subsequent cycles and completely disappears in the fourth cycle, the sorption and the desorption curves

being perfectly coincident. This interesting behaviour of calcium arabate, analogous to that of rice grain⁷ leads to the following conclusion, in accordance with the cavity concept³ which has already been established to be a general cause⁸ of hysteresis in sorption. Calcium arabate on its initial activation, has capillaries some of which are open pores and some are cavities having narrow necks. In the beginning, these cavities have rigid walls. After they are filled up, they trap the water and retain it when desorption is effected. Thus the arabate retains, for the same vapour pressure, more of water during desorption than during sorption. With progressive sorption and desorption, however, the gum swells and the walls of the cavities become more elastic. With an increase in the elasticity of the cavity wall, the cavities are less effective in trapping water. Thus after a certain stage, *i.e.*, in the fourth cycle of sorption and desorption, they have completely lost the power of trapping water, as indicated by the sorption and the desorption curves being coincident.

The hysteresis loop in the first sorption and desorption extends up to a relative humidity of 0.78. This corresponds to a maximum radius of 40.5 Å of the cavities in the sample of calcium arabate, as calculated from Lord Kelvin equation,^{4,10} $\ln \frac{p}{p_s} = \frac{-2\sigma v}{rRT}$, where p is the pressure at the concave surface, p_s the pressure of saturated vapour of liquid in bulk at that temperature, σ is the surface tension, v is the volume of one gram mol. of condensed liquid, r is the radius of the capillary, R the gas constant (8.315×10^7 ergs), T the absolute temperature, and \ln represents the natural logarithm to the base e . The fact that the hysteresis loop stretches down to zero pressure, as in rice-water⁷ system indicates that some of the cavities have necks of molecular dimensions. Compared with the activated rice grain, calcium arabate has much finer cavities. Some of the biggest cavities in rice, however, are of micro-

scopic dimensions, as indicated by the peak of the hysteresis loop extending up to the saturation point.

Dhal grain (*Cajanus indicus*), a member of the dicotyledonous seeds, exhibits similar behaviour⁹ of having a hysteresis loop in the first cycle of sorption and desorption which disappears in the subsequent cycles. A series of sorptions and desorptions of carbon tetrachloride vapour on the activated rice grain have shown the existence of a permanent hysteresis loop which has been reproduced at the ninth sorption and desorption, whereas with water, the hysteresis loop exhibited in the first cycle of sorption and desorption has been found to disappear⁷ in the third cycle.

All these observations afford a convincing proof of the view already expressed about the effect of elasticity³ of the gel system on hysteresis in sorption. It is indeed probable, that all grains and plant materials, which become elastic by virtue of their property of swelling on the imbibition of water, exhibit no permanent hysteresis loop in the sorption of water and other solvating liquids. With nonsolvating liquid, however, there is no swelling and the porous gel retains its rigidity even after a series of sorptions and desorptions. The hysteresis loop should, therefore, remain permanent and perfectly reproducible as in rice-carbon tetrachloride system.⁹ So, in all elastic gels, in the sorption of vapours of solvating liquids, as a rule, there should be no hysteresis loop. Even if there is a hysteresis loop, it should disappear after a certain number of sorptions and desorptions. Whether there is an initial hysteresis loop or not, depends upon the previous history of the gel, *e.g.*, drastic desiccation of the gel at high temperatures which may result in the production of cavities.

The investigations, *vide infra*, on a series of sorptions and desorptions of the vapours of different liquids, on a few typical organo-gels

have revealed the rôle of elasticity of organogels on hysteresis in sorption.

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¹ Bernhart, Earle Arnow and Bratton, *J. Ind. Eng. Chem., Anal. Edn.*, 1937, 9, 387.

² Briggs, D. R., *J. Phys. Chem.*, 1934, 38, 867.

³ McBain, *J. Amer. Chem. Soc.*, 1935, 57, 699.

⁴ —, *The Sorption of Gases and Vapours by Solids*, George Routledge and Sons, Ltd., London, 1932, 433.

⁵ —and Bakr, *J. Amer. Chem. Soc.*, 1926, 48, 690.

⁶ Rao, B. S., *Curr. Sci.*, 1938, 6, 446.

⁷ Rao, K. S., *Ibid.*, 1939, 8, 256.

⁸ —, *Ibid.*, 1939, 8, 468; 1939, 8, 546.

⁹ —, Unpublished.

¹⁰ Thomson, *Phil. Mag.*, 1871, (4), 42, 448.

The 'Tyrosinase' from *Dolichos lablab*

IN view of the recent work¹⁻⁵ on phenolases which has revealed conflicting views regarding the existence of distinctly separate enzymes for the oxidation of the mono- and dihydroxy phenols, it was of interest to purify and elucidate the nature of the "tyrosinase" present in *Dolichos lablab*. The enzyme extract was obtained by an extraction of the dried and powdered seeds with saline (5 per cent. NaCl). On removal of the salt by dialysis, the globulins of the extract were thrown down, which were subsequently filtered off. The resulting clear light brown filtrate containing most of the enzyme was saturated with ammonium sulphate, when the enzyme was completely precipitated. A further purification of this precipitate was effected by fractional precipitations with alcohol and acetone and by adsorption on calcium phosphate gel.

The enzyme preparations oxidise catechol with great ease, while the oxidation of phenol, *p*-cresol, tyrosine, pyrogallol and "dopa" does not proceed with the same vigour. The course of oxidation of these substrates has been followed manometrically by measuring the oxygen uptake in a Warburg. Through a series of

preliminary trials, the conditions for obtaining a measure of the activity of the enzyme preparations, were standardised. The oxygen uptake with phenol and *p*-cresol was found to be linear (after a short initial induction period) and proportional to the concentration of the enzyme. With catechol, as reported by Wagreich and Nelson,^{5,6} the oxygen uptake falls off rapidly, presumably due to inactivation of the enzyme. But the secondary oxidation of hydroquinone or ascorbic acid through the aid of the catechol as "carrier" proceeds at a rate which is a measure of the enzyme. The rate of oxygen uptake is proportional to the concentration of carrier within narrow limits (0.01 to 0.03 mgm. in 2 c.c.).

For a comparative study of the activity of the enzyme preparations towards mono- and dihydroxy phenols, the oxygen uptake with ascorbic acid/catechol and with phenol were measured. The oxygen uptake with hydroquinone/catechol and *p*-cresol also follow closely the above. The measurements were made at pH 6.2 on 1 mgm. quantities of substrate in a final volume of 2 c.c.

The following table indicates the effect of a preliminary purification on the activity of the enzyme towards the two different sets of substrates.

It will be observed that the activity towards phenol decreases as compared to catechol, with progressive purification. The induction period with phenol becomes prolonged while the oxidation of catechol begins instantaneously in every case. The addition of minute amounts of catechol (0.02 mgm.) to the phenol practically abolishes the induction period, increases the oxygen uptake, which, however, tends to fall off instead of remaining steady. In the fractional precipitations with alcohol and acetone, no other fractions of the enzyme with a comparatively greater activity towards phenol than catechol, could be obtained. The centrifugate after adsorption was also not more active towards phenol. These results suggest that the activity towards phenol (or *p*-cresol or tyrosine) is a secondary reaction depending on the