# 7091 C

## The Laxative Principle in Prunes.

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No satisfactory study of the widely recognized cathartic effect of the French or Santa Clara variety of prune has ever been reported as far as we know. The purgative action appears in foodstuffs derived from prunes as well as in the dried prune itself. The apparent thermostability of the evacuant principle may be the factor leading Sollmann[1] and others to include prunes with the colloid and emollient laxatives which act "mainly by retaining water in the intestine by imbibition, thus modifying the bulk and consistency of the feces, so that these are more easily expelled." Water-soluble substances in prunes have previously been held to play only a subsidiary rôle in catharsis; Sollmann[1] explains their action thus: "in fruits (prunes, . . . . , etc.) this colloid action is supported by the organic acids and sugars."

We studied the cathartic efficacy of the soluble compounds present in dried prunes in an attempt to isolate the active substance responsible. Since such an active principle is present in very high dilution, routine chemical tests yield little information. The color reactions described by Tumin Katti and Beal, Fuller, Warren, and others[2] for anthraquinone derivatives which act through an irritant effect, are negative according to Mrak and Smith[3] for all prune extracts tested, indicating the absence of emodin-like compounds. The $FeCl_3$ test for phenolic groups is positive.

Three methods were used to evaluate the potency of various extracts. In spite of Sollmann's criticism[1] of the Magnus technique[4] with isolated gut, it is a convenient one to determine whether or not activity is present in materials tested. Preliminary tests were made

* This effort was conducted in cooperation with W. V. Cruess, E. M. Mrak, and Clifford Smith of the Division of Viticulture and Fruit Products of the Department of Agriculture of the University of California, Berkeley, who prepared most of the extracts studied.

[1] Sollmann, T., *Manual of Pharmacology*, Philadelphia, 4th Ed., 1932, 244, 228.

[2] Warren, L. E., *Am. J. Pharm.*, 1914, **44**, 86; Beal and Tumin Katti, *J. Am. Pharm. Assn.*, 1925, **14**, 865; Tumin Katti and Beal, *J. Am. Pharm. Assn.*, 1926, **15**, 847; Fuller, H. C., *J. Assn. Off. Ag. Chem.*, 1926, **9**, 306; 1927, **10**, 364.

[3] Personal communication, 1933, confirming G. A. Pitman.

[4] Magnus, R., *Arch. ges. Physiol.*, 1904, **102**, 123.

by this technique on various prune preparations and the results so obtained were in accord with those found in intact animals by either Macht's[5] or Eddy's[6] method.

Actions of prune extracts on isolated rabbit, guinea pig, dog and cat jejunum or duodenum in either Sollmann and Rademaekers'[7] or Sollmann and von Oettingen's[8] solutions are summarized in Table I. The individual responses were confirmed at least in triplicate, and the tabulated results represent some 200 separate experiments. The water extracts were prepared according to the directions of Mrak and Cruess.[9] All other extracts were thoroughly freed of solvent by prolonged evaporation *in vacuo* and dissolved or suspended in water before being added to the bath. None of the extracts was sufficiently acidic or basic to influence the observed effect.

From the results in Table I it would appear that there are 2, and perhaps 3, different effects demonstrable. The first, which increases both tonus and amplitude of contraction, is constantly shown by an agent soluble in water, alcohol and pyridine, but which cannot be completely removed from finely ground and well desiccated prune pulp even on exhaustive extraction. This prune constituent loses its activity on extensive hydrolysis. The other effects noted with different extracts may be due to its split products which may have different solubilities, and which themselves may be present in whole prunes. A marked diminution of tonus and complete suppression of peristalsis is noted with acetone extracts. The agent involved here may be responsible for the primary inhibition of tonus and peristalsis which constantly occurs before the isolated gut shows the remarkable increase in activity on adding alcoholic or aqueous extracts to the bath. A third effect, as yet unexplained, occurs with basic lead acetate precipitates, which reduce tonus while causing an increase in amplitude of contraction. This effect is under further investigation.

Comparisons of kymograph tracings obtained by the Magnus technique from water extracts of prunes with those obtained from 16 other representative cathartics of various chemical types, show a resemblance only to diacetyl dioxyphenyl isatin ("Isacen"). Di-

[5] Macht, D. I., and Barba-Gose, *J. Am. Pharm. Assn.*, 1931, **20**, 558; Macht, D. I., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1272.

[6] Eddy, N. B., *J. Pharmacol. Exp. Therap.*, 1932, **45**, 339.

[7] Sollmann, T., and Rademaekers, A., *Arch. Intern. Pharmacol.*, 1925, **31**, 39.

[8] Sollmann, T., von Oettingen, W. F., and Ishikawa, Y., *Am. J. Physiol.*, 1928, **85**, 118; Whitehead, R. W., *Am. J. Physiol.*, 1929, **89**, 253.

[9] Mrak, E. M., and Cruess, W. V., *Univ. Calif. Ag. Exp. Sta. Bull.*, 1929, **483**, 1.

TABLE I.
Effect of Prune Extracts on isolated rabbit Jejunum or Duodenum.

| Source | Extract | Treatment | Dilution† | Effects on Tonus | Effects on Amplitude |
|---|---|---|---|---|---|
| Prune Pulp | aqueous | none | 1:5,000 | ++ | ++ |
| | | charcoal | 1:100 | 0 | 0 |
| | | filter cell | 1:5,000 | ++ | ++ |
| | | kaolin | 1:1,000 | + | + |
| | | acid hydrolysis | 1:100 | 0 | 0 |
| | | basic " | 1:100 | 0 | 0 |
| | | yeast fermentation | 1:1,000 | 0 | + |
| | | Pb acetate | 1:100 | 0 | 0 |
| | | heating (autoclave) | 1:1,000 | ++ | ++ |
| | alcoholic | none | 1:5,000 | ++ | ++ |
| | | charcoal | 1:100 | 0 | 0 |
| | | filter cell | 1:1,000 | ++ | ++ |
| | | Pb acetate | 1:100 | 0 | 0 |
| | acetone | none | 1:1,000 | — | — |
| | | acid hydrolysis | 1:500 | 0 | — |
| | | emulsin " | 1:500 | — | — |
| | | maltose " | 1:500 | — | — |
| | ether | none | 1:100* | 0 | 0 |
| | pyridine | " | 1:500 | ++ | ++ |
| | | exhaustive extraction | | | |
| | — | ether | 1:1,000 | + | ++ |
| | — | alcohol | 1:1,000 | + | + |
| | — | water | 1:1,000 | ++ | ++ |
| Charcoal Adsorbate | — | elution at pH 9.0 | 1:100 | 0 | 0 |
| of aqueous or | — | acid elution | 1:100 | 0 | 0 |
| alcoholic extracts | — | alcoholic " | 1:100 | 0 | 0 |
| | — | acetone " | 1:500 | — | — |
| Lead acetate ppt. | — | $H_2S$ to remove Pb | 1:500 | — | ++ |
| of aqueous or | — | same, of first fraction | | | |
| alcoholic extracts | | only | 1:100 | + | + |
| Alcoholic Extract | ether | none | 1:500* | 0 | ± |
| | acetone | " | 1:500 | — | — |
| Prune Pits | aqueous | " | 1:100 | + | + |

† = total prune solids / volume of bath
* = suspension

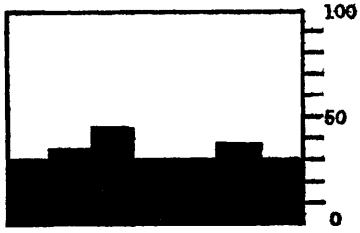
++ = marked increase
+ = definite, but smaller increase
0 = no effect
— = marked decrease

hydroxyphenyl isatin was prepared and gives the same type of physiological response with isolated gut; it also reacts with $FeCl_3$, while diacetyl di-oxyphenyl isatin does not. Chlorogenic and caffeic acids also cause an immediate increase in tonus and amplitude of contraction similar to that caused by prune extracts. These acids were tested because of the similarity in color reaction with that developed in the prune phenolic compound on treatment with $FeCl_3$.

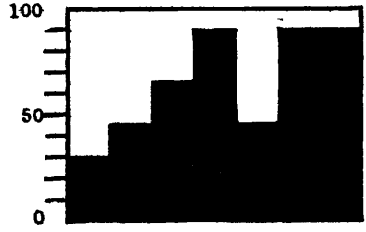
These results on the isolated gut were confirmed by experiments using Macht's method,[5] but since the observed mean deviation was high and a minimum of 10 rats had to be sacrificed for each determination, we made use of this technique for only a selected group of prune preparations. Results expressed as the average per cent of the

total length of intestine, from pylorus to anus, traversed in 50 minutes by a test meal of a suspension of wood charcoal in gum tragacanth incorporated with the extract to be tested, in groups of 10 rats, were: controls, 55 ± 6%; water extract of prunes clarified with a filter cell, 1.0 gm./kg., 63 ± 5%; pulp of the whole dried prune, 1.0 gm./kg., 66 ± 10%; and the alcoholic extract of prunes, 1.0 gm./kg., 69 ± 8%.

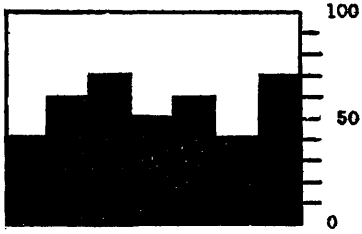
Average Effects of Prunes on Defecation in Groups of 10 Rabbits by Eddy's Method.
Abscissae: time in hours. Ordinates: percentage of animals defecating.



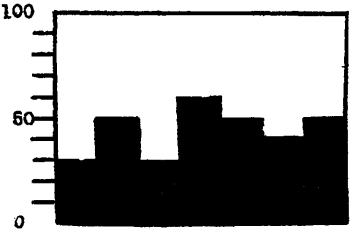

1. Average 9 control groups.




2. 3.0 gm./kg. alcohol extract.




3. 0.5 gm./kg. water extract.




4. 0.5 gm./kg. dried prune pulp.

Eddy's technique[6] was more satisfactory. Representative observations are shown in the charts of Fig. 1. The results obtained on isolated gut were again confirmed. The discrepancy between Eddy's control group figures and those in Fig. 1 is probably due to the more highly constipating diet deliberately used in our experiments.

*Summary.* As tested by the techniques of Magnus, Macht, and Eddy, the laxative properties of prunes are not due entirely to colloidal or emollient effects in the intestine, as has been previously supposed, but would seem to depend also on the presence of an agent soluble in water, alcohol and pyridine. This active principle in prunes which is broken down by strong hydrolysis has otherwise certain chemical and physiological properties similar to di-hydroxyphenyl isatin and to caffeic and chlorogenic acid.