TECHNICAL PAPERS

Citric Acid in Saliva

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Positive evidence for the occurrence of citric acid in human saliva appears to be limited to a report by Pucher, Sherman, and Vickery (*5*), who found 0.04–1.30 mg. per cent citric acid present in 7 specimens. Previously, Kuyper (*1*) and Leake (*2*) were unable to detect citric acid in saliva. However, they both used methods which at the time were unsuited to detect less than 2.0 mg. per cent. With slight modifications the method proposed by Perlman, Lardy, and Johnson (*4*) has been adapted to saliva analysis using 10-cc. quantities. Some 180 saliva specimens obtained from 15 adult men have been analyzed, and the results (Table 1) afford considerably more evidence of the presence of citric acid in saliva than has been available heretofore. Paraffin-stimulated saliva was collected on three days at four different times, *i.e.* 9 A.M., 11 A.M., 1 P.M., and 3 P.M. The figures in the table are averages for three days according to time of day for each individual.

TABLE 1

CITRIC ACID* IN STIMULATED SALIVA

(Mg./100 cc.)

Case	9 A.M.	11 A.M.	1 P.M.	3 P.M.	Average
1	0.44	1.24	1.21	1.37	1.07
2	0.73	0.55	0.85	0.51	0.66
3	0.60	0.57	0.83	0.66	0.67
4	0.79	0.89	0.87	0.72	0.82
5	0.56	0.60	1.04	0.75	0.74
6	0.56	0.56	0.64	0.68	0.61
7	0.86	0.70	1.37	1.02	0.99
8	1.42	1.94	1.95	1.61	1.73
9	1.74	2.14	2.40	1.86	2.04
10	1.19	1.13	1.81	1.51	1.41
11	0.98	1.00	1.42	1.25	1.16
12	0.88	0.92	1.31	1.11	1.06
13	0.67	0.55	0.81	0.52	0.64
14	1.48	1.49	1.56	1.61	1.54
15	1.00	1.40	1.13	1.31	1.21

* Expressed as the monohydrate.

These results support the data of Pucher, *et al.* (*5*) and indicate that the average male adult's saliva may contain 0.50–2.00 mg. per cent citric acid. In nearly every case, results of triplicate analyses were quite consistent. In addition, samples taken every two hours throughout the day were essentially consistent, except perhaps for the slightly higher values for the 1 P.M. specimens.

Further studies are contemplated, particularly on the relation of salivary citric acid to dental erosion and dental caries. Decalcification of dental tissues by citrate ion is suggested by the observation that calcium and citrate form a soluble, slightly ionized complex (*6*). Also, it has been observed in previous studies from this laboratory that citrate in practically neutral drinking fluids has a pronounced destructive action on dental tissues *in vivo* (*3*).

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Wright's Hypothesis: Its Relation to Volume Growth of Tissue Cells and Mitotic Index

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In studying the length of time spent by cells in the different stages of mitosis Wright (*6*) first stated and made use of the following hypothesis: The fraction of the total time of mitosis spent by a cell in a given phase of mitosis is equal to the ratio of the number of cells found in that phase to the number of cells found in all phases of mitosis. According to this hypothesis it is possible to determine the time duration of all phases of the mitotic process if one can observe the duration of a single phase. For example, Wright estimated that telophase required 5 minutes for chicken-heart cells incubated at 37°C. From this time and the percentages of cells found in the various phases he established the time schedule for the mitotic cycle, the total duration of which turned out to be 34 minutes.

On the other hand, the mitotic index has been used as a measure of the rate of growth of cells in tissues (*3*, *4*). This interpretation placed on the mitotic index assumes tacitly that the time required for mitosis, T, is constant. It can be shown that the mitotic index is proportional to the product of the time of mitosis, T, and the rate of cell division. For the important case of exponential growth of tissue volume such as occurs in liver regeneration (*1*, *4*) and in transplantable mouse-tumor growth (*2*, *5*) it can be shown that the mitotic index, m/M, is equal to $(e^{\lambda T} - 1)$ in a first approximation, where λ is the characteristic growth constant in the tissue volume growth law, $v = v_0 e^{\lambda T}$. Values of λ have been measured for regenerating liver: $\lambda = 1.33$ days^{-1} (*1*), and for transplantable tumors: $\lambda = 0.37$ days^{-1} (*5*). Since T is usually of the order of 40 minutes, the product λT is small, and the mitotic index is: $m/N = \lambda T$. This equation indicates the basis for Wright's hypothesis if one considers m as being the number of cells in any one of the stages of mitosis and T is the time spent

by the cell in that stage. However, λ is a measure of the growth rate and must be assumed to be constant.

The biologic variation can be taken into account by considering the "partial mitotic index," $m_i/n_i = \lambda_i T_i$. The total mitotic index becomes:

$$\frac{m}{N} = \frac{\Sigma m_i}{\Sigma n_i} = \frac{n_i \lambda_i T_i}{\Sigma n_i}. \quad (1)$$

If T_i is the same for all cells in the tissue mass, the mitotic index becomes:

$$\frac{m}{N} = \frac{T}{N}\{n_1\lambda_1 + n_2\lambda_2 + \cdots + m_r\lambda_r\}, \quad (2)$$

indicating again that the index is proportional to the product of T and the volume rate of growth of the tissue. The biological variation, *i.e.* the distribution of cells into the numbers n_1, n_2, n_3 .....can be measured by measuring the volumes of cells in early prophase. This will give a frequency distribution of volumes which, when compared with the frequency distribution of volumes of all cells in the tissue, should give a measure of the fraction of cells in the so-called "resting stage." This fraction is of interest in exponentially growing tissue masses because it indicates the possibility that the growth constant, λ, for the entire tissue mass is less than that for the cells which actually contribute to the growth. Values of λ = 1.33 days^{-1} lead to a volume-doubling time of 0.3 days. If a fraction of all cells are resting, the growing cells must double their volumes (on the average) in a time less than 0.3 days.

In the case of a transplantable mammary adenocarcinoma (*2*, *5*), for small tumor volumes (less than 0.5 cc.), the volume growth of all cells can be considered approximately equal. Here Wright's hypothesis may be generalized and applied to the distribution of cell volumes to measure the rate of growth of the tumor mass. According to the hypothesis, the number of cells, Δn, having volumes in the interval, Δv, for a time, Δt, is given by

$$\frac{\Delta n}{N} = \frac{\Delta t}{L}, \quad (3)$$

where L is the intermitotic time and N is the total number of cells. If the frequency distribution of volumes is $\phi(v)$ then $\Delta n = \phi(v)\,\Delta v$, and the intermitotic time is

$$t = \frac{L}{N}\int_{v_0}^{v} \phi(v)\,dv. \quad (4)$$

Here v_0 is the smallest volume in the distribution, and t is a function of v. Equation 4 is the inverse of the tissue growth function, $v = V(t)$, and permits the determination of volume growth by means of the distribution function, $\phi(v)$. It can be shown that for exponential growth in the ideal case the function $\phi(v)$ is of the form $1/v$. Preliminary integration of the volume distribution of cells published (*2*) shows that Equation 4 leads to a logarithmic relation between t and v with a characteristic constant, λ = 0.44 days^{-1}, where the measured value was λ = 0.37 days^{-1}. Improved techniques for measuring the cell volume distribution are being developed. The method must be tested on tumors of various ages to ascertain the possible effect of "resting cells" on the volume distribution.

It should be emphasized that this use of Wright's hypothesis applies only to a homogeneous, dedifferentiated group of cells having uniform growth rate.

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Germination and Free Fatty Acid in Individual Cotton Seeds

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Seeds from cotton that has been exposed to wet weather in the field are likely to be lower in viability and to contain higher percentages of free fatty acids than those from seed cotton harvested without unfavorable exposure (*3*, *4*). Similar observations have been made of cottonseed stored under conditions of high moisture or temperature (*4*). Conventional methods of approach to the relationship of free fatty acid content to germination would require that a sample of several hundred grams of cottonseed for the free fatty acid determination and another sample of several hundred seed for germination tests be drawn from each lot tested. When sufficient data were obtained, statistical methods could be used to study the relationship between the two variables. A second approach to the problem consists of the application of microchemical methods to the analysis for the free fatty acid content (*2*) of part of the nongerm portion of a single seed and the germination of the remainder of the seed.

In order to establish whether the free fatty acid content of the nongerm end of a hulled cotton seed was correlated with that of the germ end, 50 seeds were carefully peeled and cut approximately in half; each half was weighed and placed in a numbered, small, glass-stoppered Erlenmeyer flask. To each flask 5 ml. of petroleum ether (American Oil Chemists' Society, Specification H 2-41) was added and allowed to stand for about 30 minutes to soften the seeds. The seeds were then ground by means of a glass rod with a flattened end. Any material adhering to the rod was washed into the flask by means of an additional 5 ml. of the petroleum ether. The flasks were then stoppered and allowed to stand for about 16 hours with occasional shaking. After the extraction was completed, 10 ml. of neutralized alcohol containing m-cresol purple indicator was added and the mixture immediately titrated with 0.005 N alcoholic KOH. During the titration the effect of atmospheric carbon dioxide was eliminated by bubbling a stream of carbon dioxide-free air through the titration flask. The free fatty acid content is calculated as per cent oleic acid by multiplying the milliequivalents of alkali used by 28.2 and dividing the product by the weight

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