that give fluorophores attached to larger biological molecules than were dialyzed away from the plasma proteins.

A logical route of disposal of any nondegradable browned products that might be formed in vivo would be via the kidneys; thus, urine would be the body fluid most accessible for examination. Examination of human urine for fluorescent products is a difficult task because of the relatively large volume output and because of the large number of other very fluorescent compounds in the urine. Because rats eliminate urine that is more concentrated and of a smaller volume, these animals were chosen to study the fate of browned products introduced by feeding and injecting synthetic browned fluorophores. During the 3 hr following the feeding of browned glucose–glycine to rats, there was no evidence of fluorescent products in blood plasma or urine, which indicated that absorption of the partially polymeric material (mol wt between 700 and 1500) may not have occurred by this time. The work of Bjarnason and Carpenter (1969) suggested that carbonylamine products, of at least a protein source, in urine would probably not be of dietary origin. They found that pure protein, in which the availability of lysine had been reduced by heat, resulted in increased fecal lysine, but little urinary lysine, indicating that absorption had not occurred. In a discussion of the nutritional significance of browning damage to foods, Clark and Tannenbaum (1974) reviewed their work and that of others that showed that a decrease in nutritional value of heated protein–glucose systems could not be accounted for by loss of lysine alone. Limit peptides that these workers prepared from insulin and glucose could not be completely digested to the bound carbohydrates; this type of undigestibility could account for unavailability of other amino acids. Recently, Tanaka et al. (1975) have shown that a simple Maillard reaction product, fructose-L-tryptophan, when placed in the colon can be passively absorbed, that some hydrolysis of this simple molecule occurs in vivo, and most is excreted in the urine without being metabolized.

Injected ^{14}C-labeled glucose–glycine browned products were very efficiently removed by the kidney and excreted in the urine; therefore, one may speculate that any naturally occurring low level browned products in animals and humans may also be removed efficiently if they are in the blood, but that their low level formation does not allow their detection by fluorescence techniques in mixtures of other highly fluorescent compounds.

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On the Local Isotherm Concept and Modes of Moisture Binding in Food Products

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As mentioned in the literature it was found that application of Henderson's equation to moisture sorption data in foods and food components can be used to characterize three "local isotherms". However, the analysis of 174 sorption isotherms comprising 71 different food products indicates that, in disagreement with a published suggestion, local isotherms cannot be used to give a precise and unequivocal definition of the physical state of water in foods.

Numerous equations have been derived for describing water sorption isotherms of food materials, and Labuza (1968) and more recently Nellist and Hughes (1973) reviewed the applicability of most of them. One of the most widely used models relating water activity and amount of water sorbed is Henderson's equation (Henderson, 1952). Although in several cases a complete isotherm may be satisfactorily represented by a single pair of constants in Henderson's equation (Henderson, 1952; Agrawal et al., 1969; Iglesias and Chirife, 1976), it has been frequently observed that two or three "localized isotherms" may be distinguished (Rockland, 1957).

Rockland (1957, 1969) suggested that moisture sorption

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isotherms are composed of generally three localized isotherms (Li's) and that each of them may represent a special type of water binding. Each local isotherm can be distinguished by graphical analysis of experimental sorption data plotted according to Henderson's equation. Although for many purposes the utility of Henderson's equation would be severely restricted if three pairs of constants need to be used to define the sorption isotherm, it seems important to investigate the existence of the local isotherms and whether or not they represent a type of water binding, as suggested by Rockland (1969). For this purpose, literature data corresponding to 235 water sorption isotherms comprising 71 different food materials were analyzed.

RESULTS AND DISCUSSION

Based on the observations of Karel et al. (1955), Rockland (1957) found that in many cases, when applying Henderson's equation in the log form suggested by himself:

$$\ln(-\ln(1 - A_w)) = n \ln X + \ln k \quad (1)$$

where A_w = water activity = p/p_0, X = moisture content, percent dry basis, and n and k are parameters, the straight line indicated by eq 1 was not obtained. Instead, the experimental points generally described three straight lines, each line being identified as a local isotherm.

Rockland (1969) derived three local isotherms from moisture sorption data in gelatin and compared the intercepts of the local isotherms with discontinuities that he observed in the variation of some physical measurements in gelatin as a function of moisture content. The physical measurements included the nuclear magnetic resonance (NMR) bandwidth, electron spin resonance (ESR) saturation peak height, phosphorescence decay time, and differential free energy. Rockland (1969) suggested that "the close correspondence of critical moisture content levels, defined by Li's intercepts and physical measurements would appear to be more than a fortuitous coincidence". Accordingly, he suggested that the three local isotherms derived from moisture sorption data in foods might be related to the generally recognized three types of bound water (Labuza, 1968; Karel, 1973) which exist in foods: (1) monolayer, (2) multilayer, (3) mobile, capillary, solution. As suggested by Rockland (1969), that additional data were required to determine whether or not the Li's concept permits an accurate definition of the state of water in foods, a study is conducted here over a very wide variety of products.

According to eq 1 a plot of $\ln(-\ln(1 - A_w))$ vs. $\ln X$ should give a straight line from which the parameters n and k may be calculated. It was found that, although in several cases the results conformed satisfactorily with eq 1, a better correlation was observed when two or more intersecting lines rather than a single straight line were plotted over the experimental data. A least-squares analysis was used to obtain the values of parameters n and k for each of the local isotherms considered. Table I (Supplementary Material) shows the calculated values together with the correlation coefficient. The intercepts of the local isotherms were calculated by solving the equations which describe the intercepting sections. Table II (Supplementary Material) shows the number of local isotherms observed and the corresponding values of moisture content and water activity at the intercepting points. In the same table and for the purpose of comparison, which will be discussed later, monolayer values are shown. Monolayer values were obtained by applying the B.E.T. equation (Labuza, 1968) to the experimental water sorption data at water activities of about 0.05 to 0.40.

Table III. Number of Local Isotherms Observed in the Moisture Sorption Isotherms Analyzed

	% total[a]	
No. of Li	A	B
1	1.28	1.41
2	17.87	7.04
3	74.0	84.5
4	6.38	7.04
5	0.42	

[a] A, percent over 235 isotherms comprising various temperatures for several of the products tested; B, percent over 71 isotherms corresponding to the same number of products.

Table IV. Comparison of Li-1 and Li-2 Intersections with B.E.T. Monolayer Values (Percent over 226 Isotherms Comprising Various Temperatures for Several of the 71 Different Products Tested)

	% total
Agreement at the 10% level[a]	18.6
Agreement at the 20% level[b]	35.0

[a] Means that the moisture content at the intercepting point is equal to $X_m \pm 0.10X_m$. [b] The same but equal to $X_m \pm 0.20X_m$.

Least-squares analysis was used to obtain the monolayer value, X_m, reported as g of water/100 g of dry material. No value of X_m was reported when the corresponding correlation coefficient suggested an unreliable value.

An analysis of some of the results presented in Table II is presented in Table III. It shows the percent of sorption isotherms analyzed which may be represented by a given number of local isotherms. It can be seen that about 80% of the isotherms analyzed may be represented by three local isotherms in agreement with Rockland's (1969) suggestion. Column A means percent over total number of isotherms tested covering all the products at all the temperatures available. Column B indicates percent over 71 different products, but considering only one isotherm per product. The temperature used was 25°C, when available, or the nearest one. This separation (columns A and B) was done in order to avoid a greater influence of those products for which isotherms at several temperatures were available. Rockland (1969) also suggested that "the lower limit of Li-2 corresponds with the so-called B.E.T. monolayer", so the intersection of Li-1 and Li-2 should agree with the B.E.T. monolayer value. To test this, a comparison is made of the intercepting moisture contents of Li-1 and Li-2 with the corresponding B.E.T. monolayer values calculated as mentioned before. The results are summarized in Table IV, which indicates the percent number of sorption isotherms analyzed for which the intercepting moisture content is equal to: (a) $X_m \pm 0.10X_m$ or (b) $X_m \pm 0.20X_m$. It can be seen that for both criteria there is a lack of agreement between most of the values compared.

According to Rockland (1969), Li-3 are associated with free water whose vapor pressure is influenced by capillary forces and the dissolution of soluble constituents. In order to test this suggestion let us analyze the intercepts of Li-2 and Li-3, which are shown in Table II. Table V summarizes the intercepting water activity values of Li-2 and Li-3 for isotherms which may be represented by three sections. It can be seen that in most of the 174 isotherms analyzed, the Li-3 define a region which begins at a water activity generally well below 0.60. Following Rockland (1969), capillary forces and/or solute depression should be responsible for the depression of water activity at 0.60 and below. The depression of water activity by capillary

Table V. Ranges of Water Activity at Which Intersection of Li-2 and Li-3 Occurred

Range of A_w	% total[a] A	B
0.40	5.17	5.0
0.40-0.50	17.24	15.0
0.50-0.60	40.23	51.66
0.60-0.70	29.31	20.0
0.70-0.80	5.74	8.33
0.80-0.90	1.72	

[a] A, percent values over 174 isotherms comprising various temperatures for several of the products tested; B, percent over 71 isotherms corresponding to the same number of products.

forces is given by the Kelvin equation:

$$\ln A_w = -(2\gamma/r) \cos \theta A \quad (2)$$

where A_w = water activity, γ = surface tension of water, θ = contact angle, A = constant, and r = radius of capillary. Considering eq 2 and the capillary radius which are more probably common in foods, Karel (1973) and Loncin (1973) suggest that definite capillary effects could only be expected above 0.9 water activity.

The depression of water activity in the region defined by Li-3 could also be due to dissolved solutes. The minimum activities which can be expected from solutes which are commonly found in foods (sugars and sodium chloride) have been reported by a number of authors (Bone, 1969; Kaplow, 1970; Cakebread, 1973; Karel, 1973; Loncin, 1973). Considering those reported values, it is doubtful that dissolved solutes in foods would be able to depress water activity to values well below 0.60, as it should be if vapor pressure is lowered by this kind of effect.

In view of the above considerations, it might be concluded that it is difficult to accept that Li-3 defines a region in which capillary forces and/or solute depression are responsible for the depression of water activity.

CONCLUSIONS

Rockland's (1969) suggestion that localized isotherms represent the three accepted modes of water binding in foods looked attractive because of the recognized relationship between modes of water binding and stability of dehydrated foods (Labuza et al., 1970; Karel, 1973). However, the results found here indicate a lack of agreement between the interception of Li-1 with Li-2 and B.E.T. monolayer values. Besides, in many cases the region defined by Li-3 begins at a water activity too low to consider that moisture is present as unbound, free water as suggested by Rockland (1969). We may conclude that, although in a broad sense the local isotherms proposed by Rockland (1969) may be related to the different modes of water binding, they cannot be used to give a precise and unequivocal definition of the physical state of water in foods.

Supplementary Material Available: Table I (Henderson's parameters n and k for Local Isotherms in Foods and Food Components) and Table II (Intersections of Local Isotherms in Foods and Food Components); 44 pages. Ordering information is given on any current masthead page.

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Protein and Amino Acid Changes in Peanut (*Arachis hypogaea* L.) Seeds Infected with *Aspergillus oryzae*

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Proteins and free and total amino acids in peanuts (*Arachis hypogaea* L., C.V. Florunner) inoculated with *Aspergillus oryzae* (Ahlburg) Cohn were examined at various time intervals over an 18-day test period. Quantities of proteins from infected peanuts which were soluble in aqueous buffer declined markedly shortly after inoculation and then increased rapidly during the later stages of the test period. Gel electrophoresis showed that proteins were converted to many low molecular weight components in infected seeds. Most free amino acids from infected seeds increased to levels greater than those of uninoculated peanuts. Amino acid profiles distinguished inoculated from uninoculated whole seeds as well as from their soluble and insoluble fractions at various intervals of the test period.

It is well known that saprophytic fungi degrade dormant, dead, and decaying plant tissues to yield products which are metabolically acceptable for the maintenance of life. In storage organs such as seeds and tubers, for example, fungal invasion results in deterioration of cellular components and ultimate loss in viability (Christensen and Kaufmann, 1965; Harman and Granett, 1972; Harman and Pfleger, 1974). Several saprophytic storage fungi, including some aspergilli, produce mycotoxins which, when ingested,

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