sufficient and severally necessary conditions, and it would seem more correct, in this situation, to describe its role as such, than as permissive of the action of other factors. Lorenz (*6*), in analyzing innate behavior patterns, has used Erhlich's term *amboceptoric* in like circumstances.

The value of this preparation is that the electrolyte activity of adrenal steroids can be examined in a conscious animal in normal correspondence with its environment at strictly controlled levels of Na^+ depletion.

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Cholinesterase Activity of Human Leucocytes

The erythrocytes and the serum of blood are known to possess cholinesterase activity, but the experiments of Fegler, Kowarzyk, and Szpunar (*1*) have been generally accepted (*2*) as proof of the absence of cholinesterase in the white blood cells of man. Hines (*3*) also reports that he was unable to find cholinesterase activity in the white layer of centrifuged human blood, but he gives no details. We wish to report here experimental evidence that human leucocytes contain appreciable amounts of an acetyl-β-methylcholine chloride (Mecholyl) (*4*) splitting enzyme.

White-cell preparations were obtained from the blood of seventeen young, apparently healthy males. The method of separation selected was the flotation technique of Vallee, Hughes, and Gibson (*5*), with the exception that the flotation solution was made up with dextran instead of the albumin used by these authors. Cholinesterase activity determinations were performed according to a modification of the electrometric method of Michel, (*6*), in which the cleavage of Mecholyl identified the white-cell enzyme as being similar to the cholinesterase of erythrocytes and brain tissue (*7*). The use of this substrate also obviated the necessity for complete removal of residual plasma from the preparations.

Concomitant determinations, using Mecholyl, of the erythrocyte cholinesterase activity were performed on each sample of whole blood for purposes of comparison. Hematocrit determinations permitted the activity values to be expressed in terms of a unit volume of packed cells, thus compensating for variations in the total packed red-cell volume.

The flotation solution was made up by first dissolving 214.2 g of sodium chloride-free clinical dextran (*8*) in 900 ml water. Then 0.6092 g of solid sodium chloride was added to 100 ml of the dextran solution to give a freezing-point depression of 0.57C° and a specific gravity of 1.079. This solution is isoosmotic with plasma and has a density that is intermediate between the densities of the normal erythrocyte and leucocyte.

For each determination, 15 ml of freshly drawn heparinized blood was carefully layered onto 10 ml of dextran solution in a 40-ml test tube having an inside diameter of 25 mm. The tube was centrifuged for 10 minutes at 42 *g* and then for 20 minutes at 1500 *g*. The plasma was drawn off and the white cells that had collected at the dextran-plasma interface were pipetted into a graduated 15-ml centrifuge tube. The volume was increased to 5 ml by the addition of isotonic saline, and the cells were homogeneously suspended by inversion.

A Van Allen microhematocrit tube of known total volume and with a calibrated stem was filled to the new calibration mark above the bulb with an aliquot of this suspension and was centrifuged at 1400 *g* for 10 minutes. Some cells usually adhered to the sloping sides of the upper part of the tube; these were freed from the wall with the aid of a thin piece of wire. The tube was then returned to the centrifuge and was spun for an additional 20 minutes. In this way, values for white-cell concentrations were obtained that were expressed in terms of volume of packed white cells per unit volume of suspension. Microscopic examination of stained smears of the packed cells showed that contamination by erythrocytes was negligible.

The remaining portion of the white-cell suspension, of known volume and leucocyte concentration, was packed in the centrifuge tube by spinning for 5 minutes at 360 *g*. The cells were resuspended in 0.5 ml of saline and transferred undiluted to a 5-ml beaker for the electrometric activity determination. To the leucocyte suspension (or to 0.02 ml of whole blood in 0.5 ml of water) was added 0.5 ml of Michel's buffer No. 2 (*9*), followed by 0.1 ml of 0.22 F Mecholyl (*10*). The initial *p*H reading was made immediately, using a model G Beckman *p*H meter equipped with glass and calomel electrodes. The beaker was then placed in a water bath at 25 ± 1°C for 1 hour, after which, the *p*H was again determined. All experimental *p*H differences were corrected for nonenzymatic hydrolysis by means of accompanying blank determinations. Hematocrits of the whole blood were determined in Wintrobe tubes by spinning at 1500 *g* for 30 minutes.

Triplicate determinations were performed on 3 successive days on four subjects in order to permit an evaluation of the analytic precision. Day-to-day variations were thus included in the measure of precision. There is a wide variation among the subjects in the values for white-cell cholinesterase, but the red-cell values fall within a narrow range, in agreement with previous results. The mean leucocyte-cholinesterase activity is 0.028 Δ *p*H/hr mm^3 packed cells, with 95-percent confidence limits of ± 0.016. The standard deviation of the triplicate analyses is ± 0.014 (coefficient of variation = 49 percent), which indicates the relatively low order of precision of the individual results. Application of the F test yielded a value exceeding the 1-percent point, from which it may be concluded that the method is sufficiently precise to detect differences from one individual to another. The mean value for erythrocytes is 0.069 Δ *p*H/hr mm^3 packed cells (standard deviation = ± 0.007); the ratio of the mean activity of packed erythrocytes to the mean activity of packed leucocytes is then 2.5. Graphic comparison of the activities of the two cell types showed the absence of any systematic relationship between them.

This investigation adds another item to the long list of tissues that are known to contain cholinesterase. Further studies will be required to elucidate the function of this enzyme in the leucocytes.

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9. This is 0.006 formal in sodium barbital, 0.001 formal in KH_2PO_4, and 0.30 formal in NaCl. The *p*H is adjusted to 8.00 by the addition of 0.1 F HCl.

10. Concentrations are expressed in volume formality, F, the number of formula weights per liter of solution.

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Clinical Experiment on the Use of Sodium N-Lauroyl Sarcosinate in the Control of Dental Caries

The belief is widely held that dental caries is caused by acids formed on a tooth surface by the enzymic degradation of carbohydrates (*1*). On the basis of this concept, attempts have been made to control dental caries by reducing the availability of the sugar substrate (*2*), by making the tooth more resistant to acids (*3*) or by the prevention of fermentation. One method of control that has been suggested (*4*) makes use of enzyme inhibitors to prevent the glycolysis of the sugars. This method has been clinically tested with the use of a dentifrice containing 2-percent sodium N-lauroyl sarcosinate (*5*), a very effective inhibitor of hexokinase *in vitro* (*6*).

The test subjects were young adults from the Meredith Publishing Company, Des Moines, Iowa, and from the student bodies of the University of Miami, Florida, the University of Florida, Gainesville, and Drake University. The experiment at each geographic location was conducted independently by different investigators, although the procedures used were the same and were formulated by an over-all coordinator.

Each subject was classified in one of the following test groups. *T-1-D*: Subjects used a dentifrice containing 2-percent sodium N-lauroyl sarcosinate morning and night. *T-2-D*: The same dentifrice was used after each meal. *C-1*: Subjects used a dentifrice of their choice in the manner to which they were accustomed. *C-2-D*: Subjects used, morning and night, a dentifrice identical to that used by the T-1-D and T-2-D groups, except that sodium N-lauroyl sarcosinate was replaced by 2-percent sodium salt of sulfated glyceride of coconut fatty acids. In all groups except C-1, instructions were given for proper brushing techniques, and all subjects were issued dentifrice and brushes as needed.

Since it was expected that 30 to 40 percent of the participants in the experiment would drop out each year, the homogeneity of the groups was not determined until the experiment had been completed. Comparisons were made then from data collected before the tests among the various groups and locations in respect to distribution of age; sex; DMF (decayed, missing, filled) surfaces; DF teeth; missing teeth; caries-free surfaces; caries-free teeth; free proximal surfaces; free labial, lingual, or buccal surfaces; and free occlusal surfaces.

At the start of the experiment, the average age of the subjects was 26 at Meredith, 19 at Drake, 22 at Miami, and 20 at Gainesville. In all groups and locations, the males were slightly more numerous than the females. Although within each group the numbers of decayed, missing, and filled surfaces were consistent, the counts were somewhat higher at Meredith and considerably higher at Gainesville. Initial examinations were made on 2543 subjects. At the end of the first year of the test, there were 1883. This number decreased to 1159 subjects who completed the full 2 years of the experiment.

The increment of new carious surfaces was determined by clinical examination and radiographs, as was the involvement of teeth that had been noncarious at the start of the tests. In this regard, test subjects were compared with the controls with the data from each installation. Inasmuch as the group at Gainesville was not strictly comparable with the others

Table 1. Dental caries activity during 2-year test period.

Group	Installation	Condition at start				2-year increment of dental caries			
				Average no.		Teeth		Surfaces	
		No. subjects	Avg. age	DMF surfaces	DF teeth	$\bar{x}$	σ^2	$\bar{x}$	σ^2
C-2-D	Gainesville	41	20.2	51.6	19.7	0.76	0.49	7.51	14.55
	Miami	86	22.1	39.4	16.6	0.57	0.51	3.19	4.91
	Meredith	182	26.6	35.8	16.0	0.39	0.40	1.78	2.72
	Drake	74	18.8	34.1	16.2	0.38	0.43	1.69	3.04
	All groups	383	23.4	38.0	16.6	0.47	0.45	2.69	7.63
T-1-D	Gainesville	60	19.5	44.5	19.0	0.40	0.45	4.25	9.62
	Miami	107	21.5	37.3	16.0	0.18	0.25	1.12	2.09
	Meredith	116	26.7	37.1	16.3	0.15	0.16	0.68	0.95
	Drake	135	19.5	34.6	16.0	0.21	0.26	1.07	1.79
	All groups	418	22.0	37.4	16.5	0.22	0.26	1.43	4.10
T-2-D	Gainesville	36	20.3	55.9	20.1	0.50	1.00	4.22	15.22
	Miami	105	23.0	40.2	16.2	0.24	0.25	1.20	1.78
	Both groups	141	22.3	44.2	17.2	0.31	0.44	1.97	6.90
C-1	Gainesville	19	19.7	56.5	20.5	0.79	1.62	7.00	24.00
	Miami	82	22.1	34.6	15.4	0.62	0.83	2.71	5.48
	Meredith	86	26.6	35.0	16.1	0.44	0.48	1.92	3.18
	Drake	30	19.4	32.0	15.2	0.17	0.14	1.40	3.35
	All groups	217	23.3	36.4	16.1	0.50	0.68	2.60	7.82

Table 2. Comparison of control and test results.

Contrast	Installation	Teeth		Surfaces	
		Reduction (%)	t	Reduction (%)	t
C-2-D	Gainesville	47	2.62*	43	4.72†
vs.	Miami	68	4.45†	65	7.82†
T-1-D	Meredith	62	3.67†	62	6.55†
	Drake	45	2.07*	37	2.88‡
	All groups	53	5.88†	47	7.40†
C-2-D	Gainesville	34	1.34	44	3.73†
vs.	Miami	58	3.76†	62	7.65†
T-2-D	Both groups	51	3.83†	57	7.03†
C-1	Gainesville	49	1.74	39	2.90‡
vs.	Miami	71	4.22†	59	5.75†
T-1-D	Meredith	66	3.78†	65	6.33†
	Drake	−24	−0.40	24	1.14
	All groups	56	5.33†	45	6.02†
C-1	Gainesville	37	0.92	40	2.30*
vs.	Miami	61	3.64†	56	5.55†
T-2-D	Both groups	52	3.23‡	44	3.98†

* Significant level 0.05. † Significant level 0.001. ‡ Significant level 0.01.