75 hypertensive patients was 147.3 mEq/1, an increase of 2.5 mEq. A statistical analysis based on 400 normotensive determinations and 75 hypertensive determinations using Fisher's "t" test(15) showed the difference between the means to be significant. The value of "t" was 6.16 and that of "P" was less than .01. The standard deviation in the 400 normal determinations was 3.81 mEq/1, and the standard error 0.19 mEq/1. In the 75 hypertensive patients the standard deviation was 4.07 mEq/1, and the standard error was 0.45 mEq/1. In the frequency distribution curves shown in Fig. 1 it is obvious that there is a tendency toward an increase in the sodium values present in the serum of hypertensive individuals. Forty-three per cent of "normal" values fall to the right of the normal mean on the curve, while 66% of the hypertensive values fall in these same limits.

Twenty-four of the patients were recalled for further examination two months after the initial values were obtained. This was thought necessary in order to determine the constancy of the values previously found. Ninety-two per cent of the values checked were within plus or minus 0.5%. We believe that these deviations were within the range of technical error.

15. Fisher, Ronald, A., *Statistical methods for research workers*, 8th edition, Edinburgh, Oliver, 1941.

Discussion. The results obtained would seem to indicate that there is a tendency toward an elevation in the serum sodium concentration of hypertensive patients. Surprisingly enough, statistically this elevation was found to be very significant. These data indicate a wide overlap between the serum sodium values found in normotensive and hypertensive patients, with a definite trend for a large group of the hypertensives to have a value in the upper normal range. One might be tempted to conclude that this finding supports the concept of adrenal participation in the production of the hypertensive syndrome, and that this might offer a basis for sodium restriction therapy in this disease. However, until the significance of these findings is more clearly understood, it may perhaps be better to avoid any conclusion concerning these findings as to their therapeutic or etiologic concepts.

Summary and conclusions. A series of serum sodium determinations made on uncomplicated hypertensive patients has been presented. The range and average serum sodium values in these patients were found to be slightly above those found in a concomitant series of 400 normotensive subjects. We have intentionally avoided any conclusions at this time as to the significance of these findings.

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Apoerythein Studies. IV. Nonidentity of Salivary, Gastric Juice, and Gastric Mucosal Apoerythein Activities. (18851)

ERNEST BEERSTECHER, JR. AND ELEANOR J. EDMONDS.
(Introduced by Edwin L. Smith.)

From the Department of Biochemistry, The School of Dentistry, The University of Texas, Houston, Texas.

Previous papers in this series have reported the normal variations which occur in salivary apoerythein (intrinsic factor) levels(1) and demonstrated the unique manner in which heating affects salivary apoerythein activity (2,3). The heating of saliva at 70°C for 15 seconds results in a complete loss of apoerythein activity, but heating below 65°C *or at 75°C-100°C* results in no diminution of activ-

1. Beerstecher, E., Jr., and Altgelt, S., *J. Biol. Chem.*, 1951, v189, 31.

2. Beerstecher, E., Jr., *Fed. Proc.*, 1951, v10, 161.

3. Beerstecher, E. Jr., and Edmonds, E. J., *Science*, in press.

TABLE I. Comparison of Thermal Stabilities of Apoerythein Activity of Saliva, Gastric Juice, and Gastric Mucosal Extract.

Sample	Temp., °C	% bacterial growth* Milliunits apoerythein/5 ml medium 0.2	0.4	0.6	0.8
Human saliva	Untreated	100	25	20	0
	70°	100	110	120	140
	90°	100	75	40	0
" gastric juice	Untreated	100	35	25	0
	70°	100	160	180	220
	90°	100	160	210	225
Dog " "	Untreated	100	90	55	0
	70°	100	105	120	125
	90°	100	100	125	140
Dog " mucosa	Untreated	100	15	0	0
	70°	100	60	15	0
	90°	100	90	30	0
Hog " "	Untreated	100	85	35	0
	70°	100	85	45	0
	90°	100	85	65	0

* 0 = no growth; 100 = growth of bacteria in absence of apoerythein.

ity. Samples inactivated at 70°C cannot be reactivated by subsequent heating at higher temperatures, but samples heated above 75°C become immune to destruction at 70°C. Thermal studies with apoerythein from gastric juice and gastric mucosa now show that the activity differs from that in saliva.

Experimental. The apoerythein assay method was that described by Ternberg and Eakin(4) in which activity is indicated by the inhibition of the growth of *Escherichia coli.* Samples of human saliva and gastric juice, dog gastric juice and gastric mucosal extract, and hog gastric mucosal extract were diluted to contain approximately one milliunit of apoerythein per ml and immersed in a water bath until the desired temperature was reached. They were held at this temperature for 5 minutes, then cooled. Different aliquots of each sample were then assayed, and the results reported in terms of the percentage of bacterial growth obtained in the absence of apoerythein. Results are reported in Table I. A greater variety of temperatures was studied than is shown, but the effects are adequately represented by the data given for room temperature materials and those heated at 70°C and 90°C.

The data indicate clearly that the apoerythein activity in each of the 3 sources studied has a different thermal behavior. Gastric juice activity disappears when the sample is heated to 70°C or higher while mucosal extract activity is only slightly decreased at 70°C or higher. Mucosal activity thus resembles that of saliva which has been heated to 90°C in its ability to withstand heating at 70°C.

Discussion. Three possibilities present themselves in explanation of the data presented in this and earlier publications(2,3). The apoerythein molecule may undergo true variations in its configuration and activity upon exposure to various higher temperatures. It is also possible that at 70°C a substance is formed from salivary apoerythein which is capable of neutralizing its normal inhibitory effect in the assay. Also, some entirely foreign factor in the saliva may be activated sharply at 70°C to inactivate apoerythein, and this substance may be inactivated at higher temperatures. Attempts to exclude some of these possibilities have to date been unsuccessful in that highly suggestive evidence has been found to support each possibility.

Summary. Salivary apoerythein activity is destroyed at 70°C, *but not* at higher temperatures up to 100°C; gastric juice apoerythein activity is destroyed at 70°C *and* at higher temperatures up to 100°C; but gastric mu-

4. Ternberg, J. L., and Eakin, R. E., *J. Am. Chem. Soc.,* 1951, v71, 3858.

cosal activity *is not* destroyed appreciably at any temperature up to 100°C.

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Action of Amino Acids on Bacterial Dehydrogenases and Glycolysis. (18852)

MARTIN FORBES* AND M. G. SEVAG.

From the Department of Microbiology, School of Medicine, University of Pennsylvania.

In a previous communication(1) it was shown that in a system containing washed resting *Micrococcus pyogenes* var. *aureus* and glucose the reduction of triphenyltetrazolium was greatly accelerated by nitrogenous substances. Effects of amino acids on biological systems have been variously reported. Amino acids were found to increase markedly the motility, fertilizing power and agglutination ability of starfish sperm(2), greatly to extend the functional life span of sea urchin spermatozoa(3) and under anaerobic conditions to induce motility in these spermatozoa which are otherwise quickly immobilized by lack of oxygen(4). In these studies no amino acid utilization was reported to occur.

The effect of amino acids observed under our conditions was subjected to quantitative analysis to characterize the underlying mechanism.

Materials and methods. Micrococcus pyogenes var. *aureus* strain 1A was carried on extract agar slants kept at 4°C. The cells washed from a slant which had been incubated at 37°C for 20 hours were used as source of inoculum. A casein hydrolyzate medium containing added glucose (0.5%), tryptophan (20 μg/ml), thiamin (1 μg/ml), and nicotinamide (1 μg/ml) was inoculated with 250,000 cells/ml, and when growth had reached the middle of the log phase, the cells were collected and washed twice with 0.066 M phosphate buffer (pH 7.0). The amount of cells used in the reaction systems was determined by comparing the turbidity of the systems with a previously calibrated curve relating turbidimetric readings (filter 56) and the dry weight of cells. The glucose content of the samples removed from the reaction systems at the start and at the end of the experiment was determined by the method of Folin and Wu(5). Under the conditions of the experiment triphenyltetrazolium, formazan and amino acids did not interfere with these determinations. Lactate was determined by the method of Barker and Summerson(6). The copper-lime treatment removed glucose, tetrazolium, histidine and alanine so that these substances did not interfere with the determinations. The red formazan formed by the reduction of triphenyltetrazolium chloride was measured by the use of the method described by Kun and Abood(7). Acetone (7 ml) was added to a 4 ml sample, the mixture shaken, centrifuged, and the supernate read on the Klett-Summerson colorimeter (filter 42). The quantity of formazan was determined by reference to a standard curve relating known concentrations of tetrazolium which had been reduced with a few crystals of sodium hydro-

* Abbott Research Fellow.

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2. Metz, C. B. and Donovan, J. E., *Science*, 1950, v112, 755.

3. Tyler, A., *Biol. Bull.*, 1950, v99, 324.

4. Tyler, A. and Rothschild, Lord, PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 52.

5. Folin, O. and Wu, H., *J. Biol. Chem.*, 1920, v41, 367.

6. Barker, J. B. and Summerson, W. H., *J. Biol. Chem.*, 1941, v138, 535.

7. Kun, E. and Abood, L. G., *Science*, 1949, v109, 144.