hamster this usually increases more than 300%. The spleen, therefore, is a better index of infection. Except possibly, for the relatively low ratios for the spleens of animals on low protein diets there are probably no significant differences in organ weight ratios under the dietary conditions studied.

Work is now in progress to determine the effect of kala-azar on the liver nitrogen in growing hamsters.

Summary. Protein intake influences the course of leishmaniasis in the hamster, deficient diets leading to earlier emaciation and death. Excess dietary protein seems to favor survival.

16821

Serum Cholinesterase in Some Pathological Conditions.*

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Of the esterases in human blood, the one hydrolyzing acetylcholine into acetic acid and choline has received the most attention in recent years because of the supposed relationship of this enzyme to the physiology of nerve activity. Actually, recent work indicates that there are two enzymes involved. One, the so-called true or specific cholinesterase, is thought to be involved directly in the transmission of nerve impulses, whereas the non-specific or pseudocholinesterase has, as yet, no assigned function.

Since the activity of the latter enzyme is easily measured, and since it is present in a readily available material (serum) in hospital patients, it offers an opportunity to study variations in a basic enzyme system in a number of pathological conditions. Such variations in themselves may be of more importance when the nature and function of the enzyme itself is better understood, nevertheless these variations are a guide to alterations in the metabolism of the organ or organs producing, distributing and disposing of the enzyme.

We have confirmed the findings of those workers who have noted a great spread in the values for pseudocholinesterase in the serum of normal individuals. This may be seen in the summary of normal values in the tables which follow. This spread has confused many observers who have been unable to come to any conclusion as to variations from the normal of serum cholinesterase in numerous pathological conditions. However, such difficulties are obviated by using sufficiently large samples thus permitting statistical analysis.

Of the published information available, there is evidence to indicate a decrease in the value of the enzyme in liver damage[1–6] and in pernicious anemia in relapse.[7,8] We have observed the decrease in cases of liver damage

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1 Antopol, W., Tuchman, T., and Schiffren, A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 46.

2 Antopol, W., Schiffren, A., and Tuchman, L., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 363.

3 McArdle, B., *Quart. J. Med.*, 1940, **9**, 107.

4 Faber, M., *Acta Med. Scand.*, 1943, **114**, 72.

5 Kunkel, H. G., and Ward, S. M., *J. Exp. Med.*, 1947, **86**, 325.

6 Wescoe, W. C., Hunt, C. C., Riker, W. F., and Litt, I. C., *Am. J. Physiol.*, 1947, **149**, 549.

7 Sabine, J. C., *J. Clin. Invest.*, 1940, **19**, 833.

8 Meyer, L. M., Sawitsky, A., Ritz, N. D., and Fitch, H. M., *J. Lab. and Clin. Med.*, 1948, **33**, 189.

due to a variety of causes. Since this phenomenon has been reported comprehensively in the literature, we are not at this time publishing our data. In three other disease states, either covered not at all or inadequately by previous workers, we have made observations which we are reporting here.

Serum cholinesterase in pregnancy. Butt and co-workers[9] found in 14 women (who were either pregnant at the time or had recently given birth) that the "readings obtained in the course of pregnancy fall within the limits of variation of normal, but with two exceptions they are all below the mean found for normal females." Hall and Lucas[10] and Milhorat[11] in small series found no variation from the normal in pregnancy. Laborit and Morand[12] observed a decrease in serum cholinesterase during labour but an increase during pregnancy and Davis *et al.*[13] found the enzyme to remain normal during pregnancy. Finally, Zeller and associates[14] found no statistical deviation from normal of the enzyme in pregnancy.

In light of the above contradictory findings, we first ran a series of pregnant women at the time of delivery. The following reagents were employed in terms of the final concentration in a total volume of 3 cc:

.1 cc serum
.0017 M. sodium bicarbonate
.0034 M. acetylcholine chloride

The production of acetic acid from acetylcholine was followed at 37° by means of the liberation of carbon dioxide from a bicarbonate-carbonic acid buffer in Warburg vessels. These vessels were gassed at room temperature with a mixture of 95% nitrogen and 5% carbon dioxide. At zero time, the acetylcholine was tipped into the serum and bicarbonate mixture. Readings were taken at 20 and 40 minutes. Since the two are comparable only the latter is given in Table I.

We may calculate from Table I that the difference between the means is 15 μl. CO_2 and the probable error of the difference is 1.81 μl of CO_2 This difference is 8.28 times the probable error (a significant difference, occurring by chance only once in over 15 million trials).

In order to verify the above observations, we repeated the experiment under new conditions. This time the reagents were identical to those used by Mazur and Bodansky[15] who followed essentially the method described by Ammon.[16] In the final reaction mixture of 3 cc, the concentration of the acetylcholine was 0.015 M (pH 7.7). The enzyme was present in .5 cc serum diluted 1:10 in the $NaHCO_3$. The remainder of the procedure is the same as described above. The results for 20 minutes are listed in Table II.

The statistical significance of the data in Table II is evident from the figures on the magnitude of the differences between the means of the pregnant groups and the normal group and the probable error of these differences. If the difference between the means is 10 times the probable error of this difference, the probability of this occurring by chance is 65 billion to one.[17] It is obvious, therefore, that there is a decrease in the serum cholinesterase during pregnancy, and also at the time of delivery.

Serum cholinesterase in tuberculosis. There are a few references to serum cholinesterase in tuberculosis in the medical literature.[18,19] None, however, has a sufficiently large series

[9] Butt, H. R., Comfort, M. W., Dry, T. V., and Osterberg, A. E., *J. Lab. and Clin. Med.*, 1942, **27**, 649.

[10] Hall, G. E., and Lucas, C. C., *J. Pharm. and Exp. Ther.*, 1937, **59**, 34.

[11] Milhorat, A. T., *J. Clin. Invest.*, 1938, **17**, 649.

[12] Laborit, H., and Morand, P., *Gynecologie et Obstetrique*, 1947, **46**, 298.

[13] Davis, M. E., Si-Feng Yu, E., and Fugo, N. W., *J. Clin. Endocrinology*, 1948, **8**, 666.

[14] Zeller, E. A., Birkhauser, H., Wattenwyl, H. V., and Wenner, R., *Helv. Chim. Acta*, 1941, **24**, 962.

[15] Mazur, A., and Bodansky, O., *J. Biol. Chem.*, 1946, **163**, 261.

[16] Ammon, R., *Arch. ges. Physiol.*, 1930, **233**, 486.

[17] Pearl, R., Medical Biometry and Statistics, Saunders, Phila., 1940.

[18] de Michele, G., *Boll. Soc. Ital. Biol. Sper.*, 1944, **19**, 66.

[19] Crestol, P., Passovant, C., Benezech, C., and Dutarte, G., *Presse Med.*, 1946, **54**, 557.

TABLE I.
Serum Cholinesterase* in Normal Women and Pregnant Women at the Time of Delivery.

	No. of individuals	Age range	Mean value* in μl CO_2	Stand. Dev.
Normal	67	18-40	90	±17
Pregnant	59	18-40	75	±13

* 40 minutes at 37°C and calculated to 760 mm mercury at 0°C.

TABLE II.
Serum Cholinesterase in Pregnant and Normal Women.

Group	No. of individuals	Age range	Mean value in μl CO_2*	Stand. Dev.	Δ
Prenatal 1-6 months	48	18-40	65.9	±13.9	10.4
" 6-9 "	38	18-40	64.6	±13.0	10.8
At term	52	18-40	64.3	±19.6	9.5
Normal women	80	18-40	86.7	±19.5	

* 20 minutes at 37°C and calculated to 760 mm mercury at 0°C.

$$\Delta = \frac{\text{Difference between the means}}{\text{Probable error of this difference}} = \frac{}{\sqrt{(P.E._{M1})^2 + (P.E._{M2})^2}}$$

TABLE III.
Serum Cholinesterase in Normal People and Individuals with Pulmonary Tuberculosis.

Group	No. of individuals	Mean	± Stand Dev.	Δ
T.B. men	60	82.7	26.5	4.1
Normal men	66	94.2	19.2	
T.B. women	83	80.9	28.5	2.2
Normal women	80	86.7	19.5	

to warrant statistical analysis. We were fortunate in being able to obtain a fairly large number of sera from patients with pulmonary tuberculosis at the Olive View Sanatorium, Olive View, California. A total of 60 men and 83 women were studied. These had been previously classified clinically as follows:

	Total	Advanced	Moderate	Mild
Men	60	50	9	1
Women	83	62	16	5

Serum cholinesterase was determined by the method described above for the second series of pregnant women. The results for tuberculosis are listed in Table III.

From Table III, it may be seen that since Δ is only 4.1 in the comparison of tuberculous and normal men, the probability of this occurring by chance is only 174 to one. In the case of the women, Δ being only 2.2, the probability of this occurring by chance is 6.25 to 1. We do not feel that these figures are significant. Hence, in our series, we may conclude that pulmonary tuberculosis does not alter the serum cholinesterase values.

Serum cholinesterase in patients with neoplasms. Greenstein[20] has reviewed in a comprehensive fashion variations from normal of enzyme values in tumor bearing hosts. Although the discovery of such variations has not proved to be of diagnostic value, we agree with Greenstein as to their basic importance: "More knowledge is needed on the analytical chemistry of the cancer cell, particularly of its enzymes and coenzymes, of the non-catalytic proteins, of the sugars, nucleic acids, fats and salts—of their analytical distribution, rate of metabolic exchange and source of origin. To an equal extent, the same can be said of normal tissues; the intensive study of these tissues is bound to be helpful in research on tumors if only by stimulation, by the illumination offered by contrasting properties, and by providing new tools and approaches."

There is no previous statistical evidence of

[20] Greenstein, J. P., Biochemistry of Cancer, Academic Press, New York City, 1947.

TABLE IV.
Serum Cholinesterase in Patients with Malignant Tumors.

Site and type primary tumor	Metastasis	μl CO_2-20 min.
Squamous cell carcinoma hand	+	33.1
" " " tongue	—	63.6
" " " face	—	90.0
" " " larynx	—	58.1
Adeno-carcinoma rectum	—	63.8
" " "	+	47.5
" " stomach	—	53.8
" " "	—	50.1
" " "	—	39.2
" " "	+	56.5
" " "	+	39.2
" " "	+	53.7
" " prostate	—	33.9
" " uterus	—	52.7
Transitional cell carcinoma bladder	—	73.6
" " " "	—	68.3
" " " "	—	78.3
" " " "	—	83.7
Myeloma femur	+	54.2
Reticulum cell sarcoma	—	59.3
	Mean =	62.7
	Standard deviation =	±18.8
	Δ =	8.3

variation in serum cholinesterase in patients with neoplasms. We have studied 26 cases of men and women with a variety of malignant tumors. The reagents and methods were the same as described for pregnant women in Table II.

The Δ value of 8.3 given in Table IV is obtained by comparing the mean for the cancer group with the mean for normal women. This gives a value lower than the true one since the cancer group contains both men and women. However, even with this lower value the probability of the difference between the means occurring by chance is over 15 million to one. We must thus conclude that there is a drop in serum cholinesterase in patients with malignant tumors.

Summary. There is a significant decrease in serum cholinesterase in women in the various stages of pregnancy and in patients with neoplastic disease. There is no decrease in our series in patients with pulmonary tuberculosis.