good results from drains of this sort, as studied in the rat, warrant further study in either larger animals or on the clinical level.

Summary. Drains made of Teflon show that, in the rat, drainage from the peritoneal cavity continued through them significantly longer than through Penrose drains. It has also been shown that there were fewer adhesions secondary to the use of this type of drain than with the Penrose drains.

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Received January 12, 1954. P.S.E.B.M., 1954, v85.

Quantitative Aspects of Micro ATPase Measurements on Plasma from Chicks with Erythromyeloblastic Leukosis.* (20897)

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We(1) described a rapid micro test for estimation of the enzymatic activity of the plasmas of chicks with erythromyeloblastic leukosis to dephosphorylate adenosine triphosphate. The procedure was invaluable in the selection of plasmas of high enzymatic activity, and, consequently, of high virus content(2-4), but the results formerly observed were variable. Further studies directed changes in the micro test with resulting decrease in apparent variation and great increase in sensitivity. The vital utility of the procedure in studies of leukosis virus would appear to warrant the present report of the simple changes in the test.

Materials and methods. Procedures were identical with those already described(1) except for the volume of plasma and the constitution of the indicator solution. For the latter, there was prepared, first, a saline solution containing, in final concentration: KCl, 0.05 M; NaCl, 0.05 M; $CaCl_2$, 0.04 M; and $MgCl_2$, 0.04 M. To this solution there were added 10 ml of brom thymol blue (0.04%, Hartman-Leddon Co., Philadelphia, Pa.). Finally, 150 mg of the dipotassium (or disodium) salt of adenosine triphosphate were dissolved in the solution, and the pH adjusted to 8.2. The solution was brought to a final volume of 50 ml and parcelled in 2.5 ml volumes in tightly stoppered tubes, at −18°C. A precipitate is formed when the pH is adjusted, but it does not interfere with the reaction. However, it should be kept dispersed during all sampling of the preparation. The indicator solution remained useful for a month or more, but new preparations were made at about 2-week intervals. Residues of the solution once thawed were discarded after the day's work. The volume of plasma employed was 3 λ, which was deposited at the bottom of a long narrow tube as before(1), and 0.12 ml of the indicator solution added. From this point the procedures were the same as those previously described(1). Precision of the micro test was estimated by electrotitrimetric measurement of the liberation of acid with larger volumes of the same plasmas. This method was essentially like that described earlier(1) with the following changes: the ionic constitution of the reaction medium, in a total volume of 25 ml, was the same as that used in the present micro test, and the pH index point was 7 instead of 8. In addition, the solution of adenosine triphosphate, of which 1 ml was used to initiate the reaction, contained 6 mg of the salt/ml, together with the same salts in the same concentrations used in the reaction medium prior to the introduction of the ATP.

Results. The findings are illustrated in Fig. 1a and 1b. The liberation of phosphorus by

* This work supported by a research grant to Duke University from the American Cancer Society, on recommendation of the Committee on Growth; by a grant from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service; and by the Dorothy Beard Research Fund.

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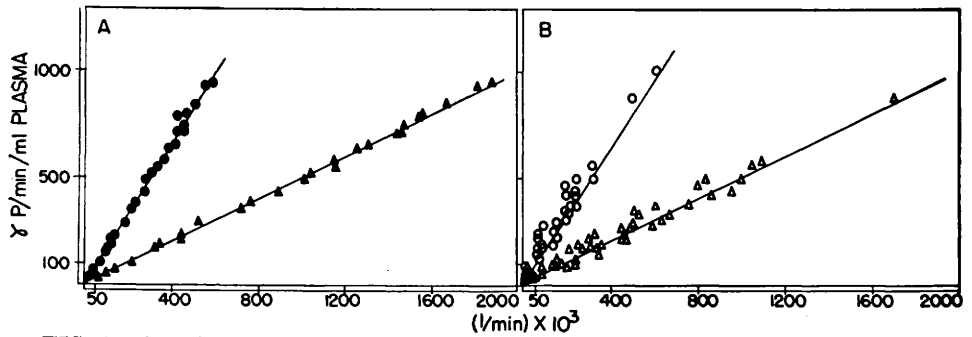

FIG. 1a. Relation of amount of phosphorus liberated from adenosine triphosphate, determined electrotitrimetrically in presence of balanced salt solution, to reciprocal of reaction time of micro test carried out with 5-λ volumes of plasma (closed circles) as previously described(1) and with 3-λ volumes (closed triangles) in presence of balanced salt solution. A single test was made with each volume on every plasma.

FIG. 1b. Micro tests with 5-λ (open circles) and 3-λ (open triangles) plasma volumes were made by the same respective technics used for experiments of Fig. 1a (on different set of plasmas) but electrotitrimetric estimations were made as previously described(1) without balanced saline solution.

26 different samples of plasma was measured as described above. In Fig. 1a values thus obtained are compared, in terms of gamma phosphorus liberated by 1 ml of plasma per minute, with the reciprocal of the time required for the indicator solution in the micro test to reach the green color of the standard solution of brom thymol blue at pH 6.6. The lower line represents the relation observed when the micro test was carried out with 3-λ volumes under the *conditions described here* and the upper line the relation obtained with 5-λ volumes of the same plasmas under the *conditions described previously*(1). It is evident that variation of individual points about the lines drawn by the method of least squares is small in both instances. The expected error of a single determination is such that in 2 of 3 cases there would be observed an error of ± 4 gamma phosphorus with 3-λ volumes of plasma and ± 7 gamma phosphorus with the 5-λ volumes. The sensitivity and resolution observed with the 3-λ volumes in balanced saline solution are approximately 3 times the sensitivity and resolution seen with the 5-λ volumes studied under the conditions of the earlier test(1).

The results obtained with 5-λ volumes in the present work, as shown in Fig. 1a, are, obviously, far less variable than those previously reported(1). An explanation of the difference is revealed in Fig. 1b which shows graphically the results of micro estimations made with a group of plasmas under conditions identical with those employed in the work described in Fig. 1a. The electrotitrimetric estimations for comparison, however, were made at pH 7 by the technic previously employed(1) *without use of the balanced saline solution.* Under these conditions the relations were highly variable whether 5-λ or 3-λ volumes were used, and the variations were of the same order as those observed before(1). It thus is apparent that at least a large part of the variations previously regarded as related to faults in the micro test were due, instead, to imperfections in the earlier conditions for electrotitrimetric estimations.

The distribution of levels of enzymatic activity in birds with the advanced disease, as shown by high primitive cell content of the blood (judged by examination of blood smears) is shown in Fig. 2 The results were obtained in a series of four routine screenings with the 3-λ volume procedure. Every bird tested is included. As already reported(1), the number of individuals with plasmas of high activity are few in number, which emphasizes, again, the value of the micro test in the selection of diseased individuals for study. These results illustrate, also, the greater range of enzyme activity susceptible to accurate measurement with the micro method now in use.

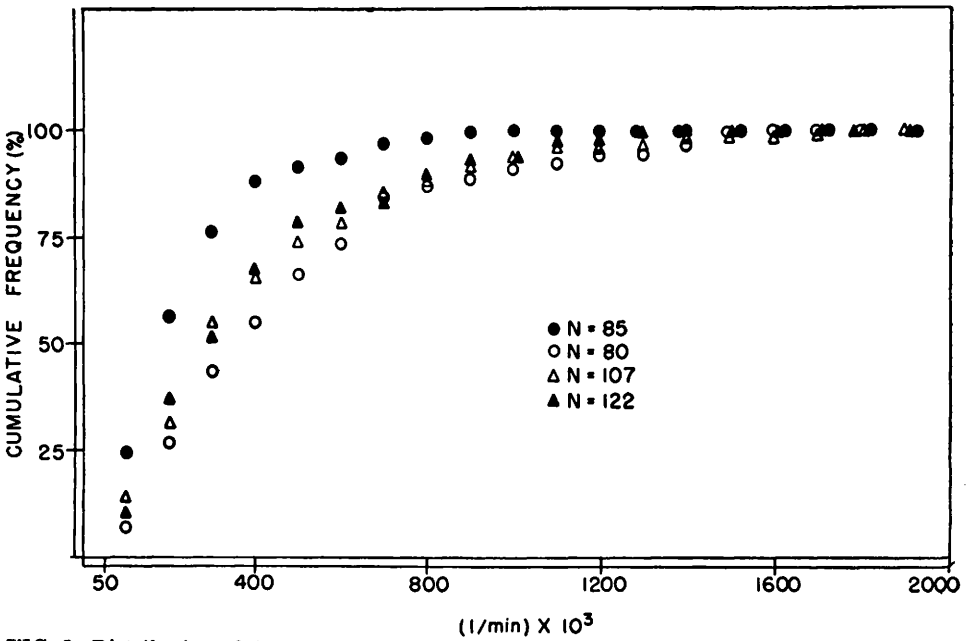

FIG. 2. Distribution of ATPase activity in 4 different groups of plasmas from birds with high primitive-cell blood content (judged by blood smears) in routine screening tests on 4 different days. N indicates number of plasmas in each group, and different symbols represent data with respective groups. All tests made with 3 volumes in presence of balanced saline solution.

Discussion. The results described here clearly demonstrate the high level of precision, sensitivity and resolution attainable with the micro method for measuring the enzymatic activity to dephosphorylate adenosine triphosphate of plasmas from chicks with erythromyeloblastic leukosis. The present work suggests that the major variations originally thought to be due to inadequacies of the micro test were related almost, if not wholly, to variations in the results obtained by electrotitrimetric measurements under unsuitable conditions. This significant improvement of the method over the procedure described earlier(1) is due, principally, to the kind and concentration of salts in the reaction mixture. It had been recognized before that both calcium and magnesium ions increased the activity of the enzyme, but the influence of a proper balance of ions in relation not only to the activity of the enzyme but to the adenosine triphosphate and to the plasma and its constituents had not been fully appreciated. Because of the greater sensitivity attained, it has been necessary to reduce the volume of plasma in the micro test to 3-λ which appears to be optimum with respect to convenience in the period required for the color change. This period is much less with the present test, a point of much importance in routine examinations of large numbers of plasmas.

Summary. Changes have been made in the conditions of the micro test of plasmas from chicks with erythromyeloblastic leukosis for their capacity to dephosphorylate adenosine triphosphate. The principal modification has involved use of a balanced saline solution in the reaction mixture resulting in a very large increase in the precision, sensitivity and resolution of the procedure. With 3-λ of plasma, instead of the 5-λ volumes employed earlier, the expected error of a single determination in 2 of 3 cases is ±4 gamma phosphorus on the basis of measurements by electrometric titration. The greater sensitivity has extended the range of plasma activity possible for accurate measurement and has shortened the time necessary for the estimation.

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Received January 14, 1954. P.S.E.B.M., 1954, v85.

Substitution of α-Keto Acids for Five Amino Acids Essential for Growth of the Rat.* (20898)

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The concept of the indispensability of the "essential amino acids" was modified when Rose(1) pointed out that the amino acids, leucine and isoleucine(2), as well as histidine (3,4), tryptophan(5,6), and phenylalanine (7) could be replaced in the diet by the corresponding keto or hydroxy acids. Subsequent studies have extended this list to valine(8-10), methionine(11), and lysine†(12) derivatives. In all of the work cited above, the growth tests were carried out with a single keto acid; the remainder of each diet was adequate with respect to all other essential amino acids. The present study was performed to determine whether 5 essential amino acids could be synthesized simultaneously by young rats at a rate which would permit growth. When leucine, isoleucine, valine, phenylalanine, and methionine were replaced in the diet by the corresponding keto acids, slow growth of the rat was obtained.

Experimental. Dimethylpyruvic acid was prepared by the method of Ramage and Simonson(15) by the condensation of acetone with hippuric acid in the presence of sodium acetate and acetic anhydride. The intermediate, 2-phenyl-4-isopropylidine-5-oxazolone, was hydrolyzed with acid and the keto acid was isolated as the sodium salt. It was characterized by its 2,4-dinitrophenylhydrazone derivative, m.p. 191.5°. *Phenylpyruvic acid* was prepared according to Organic Syntheses (16). It was isolated as the free acid, m.p. 150°. The 2,4-dinitrophenylhydrazone melted at 188°. *α-Ketoisocaproic acid. N*-chloroacetyl-*DL*-leucine was prepared according to the method of Doherty, Tietzmann, and Bergmann(17). The acyl amino acid was heated with acetic anhydride and a trace of pyridine for 3 hours at 60-70°. The mixture was fractionally distilled *in vacuo* and the 2-methyl-4-isobutylidine-5-oxazolone collected between 65-70° at 0.05 mm Hg. It was hydrolyzed to α-ketoisocaproic acid by boiling with *N* hydrochloric acid for 3 hours. The keto acid was extracted with ether, and the ether extract was concentrated to an oil which was dissolved in ethanol. The sodium salt of the keto acid was precipitated by addition of alcoholic sodium hydroxide. The melting point of the 2,4-dinitrophenylhydrazone was 156° (18). *α-Keto-β-methylvaleric acid.* A mixture of *DL*-isoleucine and *DL*-alloisoleucine was converted to the corresponding chloroacetyl derivatives. These were converted to the keto acids by the procedure outlined above for α-ketoisovaleric acid. The *DL*-keto acid was obtained as the sodium salt. *α-Keto-methylthiobutyric acid.* The method of Cahill and Rudolph(11) was modified. *DL*-methionine was converted to an oily chloroacetyl derivative by the action of chloroacetyl chloride and sodium hydroxide at −5°. The chloroacetylmethionine was treated with acetic anhydride and a trace of pyridine. A spontaneous reaction occurred. After 20 minutes the solution was fractionally distilled and the product, 2-methyl-4-(β-methylthioethyl-

* This study was supported in part by a grant from the Nutrition Foundation.

† Since lysine(13) and threonine(14) are not synthesized from the "nitrogen pool", these amino acids fall into a special category and need to be investigated further.