mercury, uranyl nitrate, and potassium dichromate(7). The glomeruli after chloroform exposure appeared to be resistant, and the cells of the macula densa were preserved, even when the rest of the cells lining the tubule at the same level were necrotic. In the animals which survived, it seems probable that a sufficient portion of the kidney remained unaffected to maintain life, since very little evidence of regeneration was found. The fate of the glomeruli after destruction of the tubules is uncertain but many appeared to survive, even though all tubules in an area had undergone necrosis or calcification.

No exact relationship was found between the areas of initial necrosis with later calcification, and the localization of alkaline phosphatase in the tubular epithelium which has been studied in sections from kidneys of normal mice(1). The cuboidal epithelium lining Bowman's space, for example, is heavily blackened when Gomori's technic is used to determine alkaline phosphatase yet almost no necrosis and calcification occurred at this site. This observation supports the studies of Hepler and Simonds(7) in dogs in which phosphatase did not appear to be concerned with calcification in the kidneys.

The alteration produced by chloroform should be useful in studies of kidney damage and repair, as suggested by Eschenbrenner and Miller(3). The sex difference offers an especial opportunity to study further the effect of hormonal alterations upon the injury to the kidney produced by chloroform as observed in the accidentally exposed $C3H_f$ males bearing stilbestrol pellets.

Summary. 1. Exposure of strain C3H mice to air containing approximately 5 mg of chloroform per liter for one, 2, or 3 hours resulted in lesions of the kidneys of all of the males but in none of the females. Histologic details of this lesion were described. 2. The lesion found in the male animals in this experiment was similar to that exhibited by the males of strains C3H, $C3H_f$, A, and HR, some of which died immediately after an accidental exposure to chloroform and some in the months following the exposure. 3. A genetic difference was apparent in that whereas strains C3H, $C3H_f$, A, and HR were susceptible, strains C57BL, C57BR/cd, C57L, and ST were resistant to the accidental exposure.

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Screening of Chicks with Erythromyeloblastic Leukosis for Plasma Activity in Dephosphorylation of Adenosine Triphosphate.* (20390)

E. B. Mommaerts, Dorothy Beard and J. W. Beard.

From the Department of Surgery, Duke University School of Medicine, Durham, N. C.

Dephosphorylation of adenosine triphosphate by the blood plasma of chickens diseased with erythromyeloblastic leukosis has been described(1). In qualitative experiments, it

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appeared likely that this activity was related to the presence of a particulate component (2,3) which has been judged to be identical with the etiological virus of the condition. This possibility has been greatly strengthened by the results of quantitative experiments currently in progress which show, unequivocally, an intimate relationship between enzymatic activity, particle number and the infectivity of the plasmas from diseased chicks.

The occurrence of the enzyme suggested the development of a rapid micro test for the screening of diseased birds for individual plasmas of high activity. A simple procedure, designed and employed routinely for this purpose, is described in this report.

Materials and methods. The origin of the virus and the characters of the disease under study have been described(4,5). All of the present results were obtained with chicks of a special strain of White Leghorns (strain 15 (6)) obtained from the U. S. Regional Poultry Research Laboratory, East Lansing, Mich. The chicks tested were from various routine titration experiments in which they had received filtered plasma from previous passage birds given intravenously at 3 days of age. *Selection of the chicks* for the test was made on the basis of the number of circulating primitive cells as judged by examination of blood smears(4). Blood was obtained by needle prick of the wing vein and drawn into capillary tubes 0.8 to 1.1 mm I.D. and 68 mm long (Kimble Glass No. 46486). Clotting was prevented by the film of heparin ("liquaemin", Roche-Organon, 1 ml = 5 mg) left in the tube by drawing in the liquid and expelling it immediately before the sample was taken. The blood was allowed to flow away from the end of the tube which was then sealed in a flame. The cells were sedimented in a refrigerated centrifuge at 1029 x g for 15 minutes. This was done with special holders, each made from an aluminum cylinder 53 mm long and 17 mm diameter and fitting a standard centrifuge cup. A set of 15 holes 38 mm deep was bored in one end to take the capillary tubes, a space being left as index point, and a handle was fitted to the cylinder for insertion and removal from the centrifuge cup. When the *cells had been sedimented,* measurements of the relative plasma, primitive cell and red cell volumes were made with a mm scale which provided an approximate estimate of the hematocrit value. The glass was scratched about 5 mm above the white cell layer, the tube was broken and a sample of 5 mm^3 (λ) was taken with a fine pointed pipet(7). This was carefully deposited at the bottom of a glass tube 100 mm long and 6 mm I.D. The tubes must be entirely free of acid or base. An *indicator solution* was prepared by dissolving in distilled water the disodium salt of adenosine triphosphate (Pabst Laboratories, Milwaukee, Wis.) in a concentration of 3 mg per ml. The pH was adjusted to 8.2 with 0.1 N KOH. For each ml of this solution, 0.2 ml of brom thymol blue (0.04%, Hartman-Leddon Co., Philadelphia) was added. It was convenient to prepare the solution in 25 ml lots and to store aliquots of 3 ml frozen. The solution could be kept in this manner for several months. Thawing and refreezing of the contents of a tube from day to day could be repeated about 3 times without destroying the usefulness of the preparation. To each tube containing 5 λ of plasma, 0.12 ml of the indicator solution was added; the mixture was frequently shaken during the reaction, and the time was recorded for the reaction to progress to the point where the color of the test mixture matched that of a control preparation of brom thymol blue of pH 6.6.

It was desirable to learn the accuracy of these results by comparison with those obtained by titrimetric measurement of the acid liberated during the enzymatic dephosphorylation. The method employed was that of Green and Mommaerts(8). For the titrimetric determinations, blood was taken in chilled, pointed centrifuge tubes by heart puncture or section through the sagittal sinus. Clotting was prevented by the presence of heparin, 1.0 mg per 10 ml blood, and separation of the cells from the plasma was accomplished by centrifugation first at 1029 x g for 15 minutes and then by spinning the resulting plasma in celluloid tubes at 3715 x g for 15 minutes. Great care was taken in pipetting the plasma after the second centrifugation to avoid the few residual cells. Plasma samples of 0.05 to 0.2 ml were used for the determina-

tion. A volume of 0.02 ml of 1.0 M $CaCl_2$ was added to a volume of Ca-free Ringer solution calculated to make, together with the amount of plasma used, a total volume of 19.0 ml. To this the plasma was added, and the mixture was incubated 3 minutes at 30°C after which 1.0 ml of adenosine triphosphate was poured in. The adenosine triphosphate solution, at pH 9, contained 4 mg of the disodium salt per ml. From this point, following the procedure of Green and Mommaerts (8), the rate of the reaction was observed by pH measurement, with glass electrodes and Beckman pH meter, during successive additions of 0.05 ml portions of 0.01 N KOH to a total volume of 0.3 ml, using pH 8.0 as the index point. Individual points in the rate line were expressed in terms of the time needed for the neutralization of each aliquot of base. By appropriate calculation, these data were transformed to an expression of the rate of liberation of phosphorus by unit volume of plasma. In a few experiments, the results obtained by this procedure were checked and proved to be identical with those employing the direct determination of phosphorus with the method of Berenblum and Chain(9). A 5 λ sample of the same plasma was taken for the final estimation with the micro test.

Results. The micro screening test described above has been employed routinely for the past 4 months, and the results obtained with the plasmas from 1128 chicks have been tabulated for the present report. Under the conditions indicated, the time required for the reaction to reach the end point has varied from 1.5 minutes, in rare instances, to periods of 15 minutes and longer. In many cases there was little or no evidence of any reaction. Observation was terminated at the 15-minute point, since, except for special purposes, plasmas of activity lower than that needed to complete the reaction within this period were of no value in the experiments for which the screening was done.

The distribution of reaction times of the plasmas under study is shown in Table I. With approximately 50% of the plasmas, the end point of the reaction was not reached within the arbitrarily chosen period of 15 minutes. The percentages of plasmas giving the end point within the 15-minute period were essentially the same for the 5-, 10-, and 15-minute times.

TABLE I. Time Required for Individual Plasmas from Chicks with Erythromyeloblastic Leukosis to Lower the pH to the End Point of 6.6 in the Micro Test.

	Total	Time of reaction—min. <5	<10	<15	>15
No. plasmas	1128	176	215	184	553
%	100	15.60	19.06	16.31	49.03

A comparison of the results of the micro test with those in the accurate determination of the enzymatic activity of the plasmas in dephosphorylating adenosine triphosphate is shown graphically in Fig. 1. These 50 plasmas were selected by preliminary micro test to afford a good distribution over the range of the most active specimens to others in the lowest range accessible to study with the volume, 5 λ, employed. In making the observations indicated in Fig. 1, the micro test was repeated on the centrifuged plasma used for titration measurement. It is evident that the relation of the reciprocal of the reaction time estimated in the micro test is linear with the phosphorus liberated as measured by titration. Variation in the results of the micro test, with respect to the titration estimations, seems uniform throughout the range. The area enclosed within the discontinuous lines represents a fiduciary band indicating the limits of the expected error of a single determination by the micro method. Thus, in such a single determination there would be expected in 2 of 3 cases an error of ±75 gamma phosphorus when the conditions of measurement are maintained as described. Since the absolute error is uniform, the percentage error becomes progressively smaller in the range of higher plasma activities.

The relation of the primitive cell content of the blood to the enzymatic activity of the plasma observed in the micro test is shown in Table II. It should be emphasized that the measurements were only approximate. The line of separation between the red and the white cells was never clear cut, and in many instances the relative volume of red cells could only be a matter of guess. It is likely, nevertheless, that the data of Table II are adequate

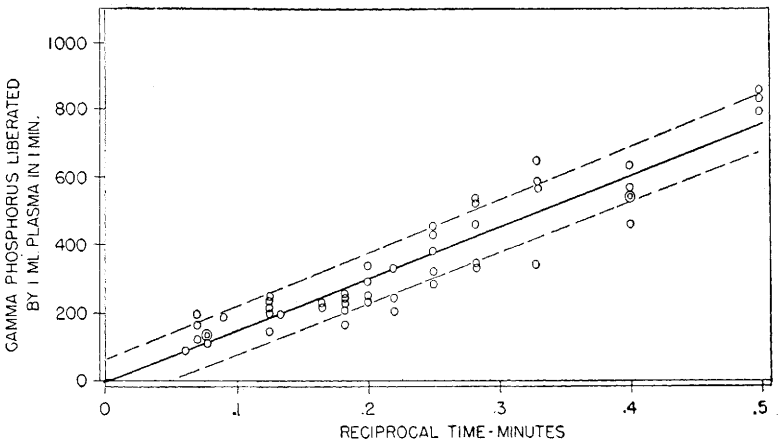

FIG. 1. Relation of liberated phosphorus measured by titrimetric determination to reaction time found in the micro test.

in revealing the essence of the relation. In the results with 609 specimens of blood, the activity of the plasma is related statistically to the concentration of primitive cells. However, it is likewise evident that the variation in the activity of the individual plasmas about the mean is enormous and overlapping. The individual variations cited in Table II indicate the limits to be expected of the results of 2 plasmas of 3 tested. The most that can be said about the relation is that while high activity is never present in the plasma of a blood of low primitive cell count, a high cell concentration is not a criterion of a high activity.

Discussion. The usefulness and ultimate application of the micro test described here, or improved modifications of it, are dependent on a number of factors. In routine work with the virus of erythromyeloblastic leukosis which is dependent on access to plasmas of high particle content and infectivity, the procedure is of much value. The particle content of the plasmas varies from about 10^9 per ml in "15-minute" plasmas to more than 10^{12} in those of 1.5 to 2-minute reaction times. This has been clearly established in an adequate series of experiments to be reported elsewhere. It can be said also that the results of the micro test provide an index of the range of infectivity of the plasma. The variations in the degree of correlation between dephosphorylation of adenosine triphosphate and infectivity are not known as well as those between particle content and enzyme activity since the number of titration experiments has been limited because of practical reasons. Nevertheless, in every instance thus far tested, high infectivity has been associated with high enzymatic activity. When it is desirable to select plasmas of high particle count and infectivity, the micro test makes possible the rapid elimination of approximately 80 to 90% of the specimens which might be regarded as useful by any other criterion. In principle, the test is applicable to the study of erythromyeloblastic leukosis in the same way as the hemagglutinative reaction is to the investigation of the influenza virus. The test possesses the advantages of much simpler application for semi-quantitative data which are probably more accurate than those obtained with hemagglutination by the

TABLE II. Approximate Hematocrit Values, in %, of Blood Samples in Relation to Time Periods Required for the Reaction to Reach the End Point in the Micro Test.

	Time of reaction—min.			
	<5	<10	<15	>15
No. chicks	107	108	93	301
RBC, Mean	11.3	11.5	13.8	16.1
WBC, "	46.5	42.5	36	23.5
S.D. of mean	±1.09	±0.97	±1.41	±1.56
Individual variation*	35–57	32–52	22–50	0–49
Plasma	42.2	46	50.2	60.4

* These values represent the limits in variation of results to be expected with 2 of 3 plasmas tested.

influenza virus. This cannot be judged finally since comparable data are not available for the hemagglutinative reaction. The fact that the absolute relationship of the enzymatic activity to the particle number and to infectivity is not fully understood does not detract from the usefulness of the test.

Use of the smallest possible volumes for the test is necessary since the chicks yielded on bleeding about 1.5 to 2 ml of plasma and even a minute drain on the circulating blood frequently leads to death of the chick before the test can be completed. It happens that with the capillary tubes employed, the volume of plasma from the best chickens was only 5 λ or 10 λ. The range of activity measurable could be extended by the use of 10 λ volumes, but in practice this has not seemed desirable.

Summary. A micro procedure has been designed for the rapid testing, in 5 λ volumes, of the plasmas of chickens with erythromyeloblastic leukosis for their capacity to dephosphorylate adenosine triphosphate. Since this enzymatic activity is related both to the number of virus particles and to the infectivity of the plasmas, the test provides the means either for the measurement of those qualities or for the selection of chicks possessing the qualities in the desired degree. There is a wide variation in this enzymatic activity among the individuals of the diseased chick population. For practical application to the study of erythromyeloblastic leukosis, the test is comparable with the hemagglutinative reaction with the influenza virus. The test in its present form would not be useful in the diagnosis of erythromyeloblastic leukosis which can be accomplished much more easily by blood smear. No experiments have been made to learn whether the reaction might occur with the plasma of birds with visceral lymphomatosis which is much more difficult to diagnose in the intact bird.

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Tubeless Gastric Analysis by Use of Insoluble Salts with Exogenous Ions.* (20391)

HARRY L. SEGAL, AND LEON L. MILLER. (Introduced by William S. McCann)

From the Departments of Medicine and Biochemistry, the Strong Memorial, Rochester Municipal and Genesee Hospitals, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Tubeless gastric analysis was introduced by Segal, Miller and Morton(1). The principle was based upon the appearance in the blood, urine and/or saliva of a non-toxic exogenous indicator ion after its release from an insoluble indicator compound by the action of the hydrogen cations of gastric juice. It was also suggested that the special indicator ion might be a dye, a radioactive material or any easily detectable substance. Extensive experience with the oral administration of an insoluble indicator compound† comprised of a cation exchange resin with the quininium cation as the specific exogenous indicator ion, has proved the efficacy of this technic(2-6). *In*

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† Marketed as Diagnex by E. R. Squibb & Sons, New York.