adrenal cortical hormones(7,8), but it is not clear whether these possible functions of ascorbic acid are related to its alliance with the eosinophils.

Summary. The action of ascorbic acid on the leukocyte response of normal (sham-adrenalectomized) and adrenalectomized rats was investigated. The data indicate that, of all the leukocytes studied, only the eosinophils are related to the action of ascorbic acid. The vitamin alters the eosinopenia of stress in intact rats, and delays the eosinophilia characteristic of stress in adrenalectomized rats.

7. Giroud, A., Martinet, M., and Bellon, M. T., *Compt. rend. soc. biol.*, 1941, v135, 514.

8. Giroud, A., and Martinet, M., *Comp. rend. soc. biol.*, 1941, v135, 1344.

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A Continuously Recording Scanning Device for Radio-Chromatographic Analysis of Labelled Antigen-Antibody Reactions.*† (18715)

ROBERT R. WILLIAMS AND ROBERT E. SMITH. (Introduced by George Brecher)

From the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda. Md.

This communication describes an application of paper chromatography to the study of interactions of antigen with antibody and a method of continuous recording to indicate the distribution of radio-isotopically labelled components. Castaneda(1) has described some reactions of Brucella antigens with their antisera on paper chromatograms using hematoxylin stained material to indicate movement or fixation of the antigen by antisera. By this means he developed a qualitative test for the presence of brucella antibodies in human sera.

Using bovine serum albumin isotopically labelled with I^{131} by the method of Knox and Endicott(2) we have attempted some quantitative measurements of antibody titre to this antigen. Also it has been possible to observe the chromatographic behavior of soluble complexes formed by antigen antibody interactions in the region of antigen excess.

Chromatographic methods. The technic described by Franklin and Quastel(3) for the chromatography of protein mixtures was adapted to the chromatography of varying volumes of I^{131} labelled bovine serum albumin with a standard volume of antiserum using aqueous buffer and salt solutions of suitable pH and ionic strength. The capillary ascent technic of Williams and Kirby(4) using Whatman No. 1 paper strips ¾ inch wide has been used throughout. The antiserum spots usually 10 or 20 mm^3 were placed with calibrated hemoglobin pipettes at marked starting points 2 inches above the lower end of the paper strip. The desired volume of I^{131} labelled antigen solution was superimposed on the antiserum spot by delivery with a Linderstrom-Lang-Holter microburette. The volume usually ranged from 1-20 mm^3. In order to achieve more uniform distribution of the antigen over the antiserum spot a small drop of isotonic saline solution was placed near the tip of the microburette and allowed to wash down the tip of the burette to the paper. There the drop of saline solution

* The technical assistance of Miss Martha C. Havens is gratefully acknowledged.

† The authors are indebted to Dr. William C. Knox for providing the standard precipitin curve on the antiserum tested.

1. Castaneda, M. R., PROC. SOC. EXP. BIOL AND MED., 1950, v73, 46.

2. Knox, W. C., and Endicott, F. C., *J. Immunol.*, 1950, v65, 523.

3. Franklin, A. E. and Quastel, J. H., *Science*, 1949, v110, 447.

4. Williams, R. J. and Kirby, H., *Science*, 1948, v107, 481.

radially chromatographed the antigen over the antiserum spot. This was particularly useful when the antigen solution volume was smaller than the antiserum volume. In order to prevent drying of the spots the paper strips were immediately placed in the jar containing the solvent. There they were suspended above the solvent for varying lengths of time before development in order to allow time for the antigen-antibody reaction to proceed. Then the lower ends of the strips were lowered into the solvent and developed for periods varying from 2-6 hours. In the experiments presented 0.15 M sodium chloride was used as the solvent.

Measurement of radio-activity. Radiochromatography has undergone rapid evolution in recent years; most methods however have employed segment analysis either by dissection of the chromatogram or radio assay of successive segments along the strip(5). It is believed that an essential improvement of these practices has been achieved by use of a continuous recording instrument.

The measuring device (Fig. 1) consisted of an end window Geiger counter and collimating slit coupled to a counting rate meter and strip chart recording milliameter. In routine procedure, 8 or 10 of the chromatographic strips were taped end to end and rolled onto a feeder spool. After passing the leading end of the strip through the slit guide and taping it to the recorder chart, a continuous record of the radioactivity distributed along the strips could be obtained upon actuation of the chart drive and counter circuits. With the arrangement described, specifications as to chart drive speed and optimal slit width are contingent upon the time constants‡ of the Geiger counter rate meter. These vary in different circuits and sensitivities from values of 1 second to about 1 minute and are minimal for the highest count rate sensitivity range in a given circuit. It is therefore expedient to

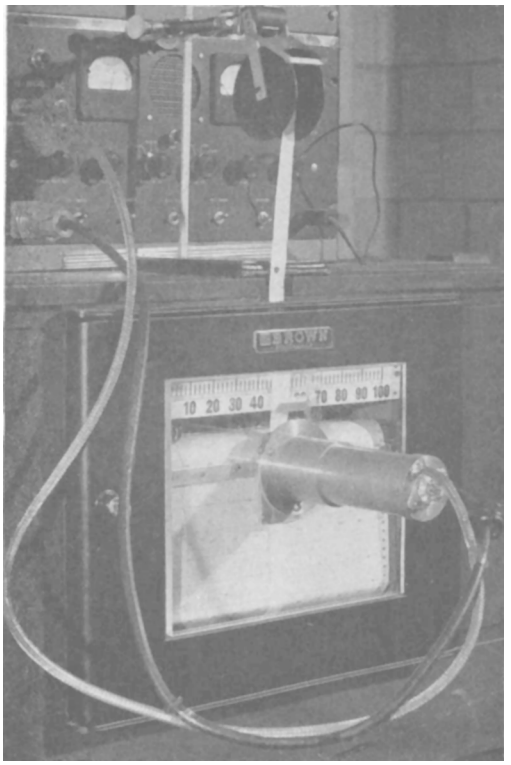

FIG. 1.
Measuring and recording assembly with chromatograms in scanning position.

work wherever possible with a high level of radioactivity in order to minimize the time constant of the rate meter response. When this is done the practical limiting factors are the chart drive speed and slit width.

In order to allow time for the rate meter to respond fully (98%) to a given small segment, $\Delta \chi$, of the strip, it is necessary that this segment remain within the slit space for a period of at least 4 times constants. The limiting slit width, S, is thus definable as the product of the chart drive velocity, V, and a time equal to 4 time constants. That is, $S = V(4T)$, where T is the time constant. On the basis of this expression Table I indicates the minimal slit widths compatible with a 98.1% response for various strip velocities at the two sensitivities used in experiments reported here.

The essential validity of these limits was confirmed in experiments with 1 and 10 mm slits at various strip velocities, and the results reported here have been held well within them.

5. Lindberg, O. and Hummel, J. P., *Ark. Kemi.* 1949, v1, 17.

‡ The "time constant", T, is ordinarily taken to be the time, t, required to reduce the exponent to unity in the expression, $A_t = A_0(1-e^{-kt})$, whereupon in one such period the response height $A_t = 0.63\ A_0$, or 63% of maximal response.

TABLE I. Slit Widths (mm) Required for 98.1% Full Response for Various Strip Drive Velocities and Response Time Constants.

Strip velocity (inches/hr)	Strip velocity (mm/sec.)	Time constants 2.5 sec.	Time constants 1.0 sec.
30	.21	2.1 (mm)	0.85 (mm)
60	.42	4.2	1.79
90	.64	6.4	2.54
120	.85	8.5	3.39

One additional factor often favors a wider than minimal slit width, namely the statistical advantage of the higher total count thus obtained, and the corresponding reduction in the standard error of measurement. Moreover, as the initial spot of active material placed on the strip is several millimeters in diameter, the ultimate precision of measurement of the gradient along the strip cannot reasonably exceed this order of error; hence a slit width of about half the spot diameter is probably a practical minimum to be sought in this work.

In Fig. 2 are recorded the radioactivity profiles obtained from a series of labelled albumin solution spots placed on a strip of Whatman No. 1 paper with the Linderstrom-Lang-Holter microburette. The areas under the curves were measured with a planimeter and are plotted in Fig. 3 as area in cm^2 against volume delivered to the paper. The volumes ranged from 1.0 to 10.0 mm^3 of a solution containing approximately 3.0 mg/ml of I^{131} labelled bovine serum albumin; the total iodine content was seven atoms of iodine per mole of albumin. Thus the calibration curve represents the range 3.0 to 30.0 μg of protein and appears very nearly a straight line up to 9.0 mm^3. It furnishes a measure of the labelled protein in a circular area with a precision of about 4% for a 1 mm slit and of 2% for a 1 cm slit. It should be pointed out that activity which has been chromatographed along the paper cannot be measured with the same precision as a circular spot except as a linear integral of a constant strip width. Results with a biuret color reaction indicate that the width of the protein spots in the upper part of the strip after development show some variation, so that the geometry of counting is less precise.

Results. In Fig. 4 are shown some radiochromatograms of I^{131} labelled bovine albumin under various conditions. Frame 1 shows that the labelled albumin in 0.15 M sodium chloride solution forms a long heterogeneous streak similar to that found by Franklin and Quastel(3). Similar results were obtained with labelled albumin in a phosphate buffer having a pH of 7.4 and ionic strength of 0.05 to 0.20. However in Frame 2 the activity from 5 mm^3 of labelled albumin solution superimposed on 20 mm^3 of normal rabbit serum is seen moving as a homogeneous single peak and by simultaneous biuret color reaction can be shown to move in the leading portion of the serum protein biuret spot. Little or no activity remains at the starting point of the chromatogram. The R_f value of the activity peak was found to be 0.95.

The different behavior of labelled bovine serum albumin chromatographed alone and

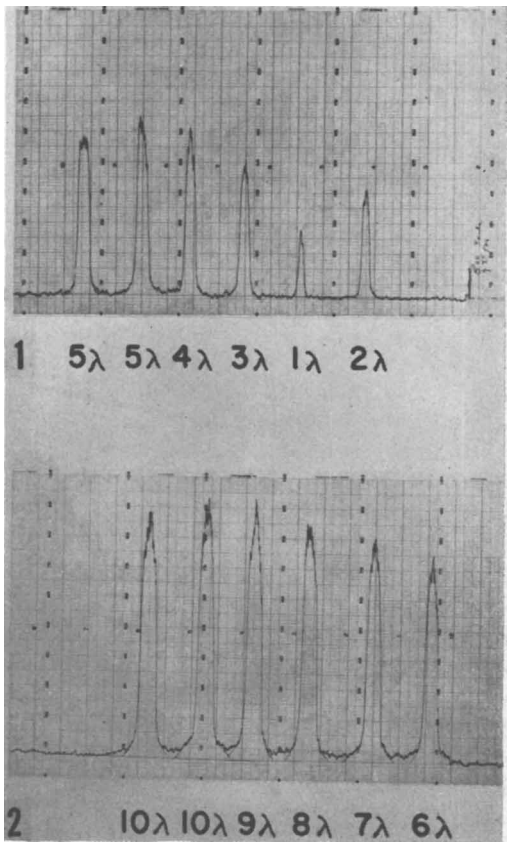

FIG. 2.
Radioactivity profiles of a series of volumes of a labelled bovine albumin solution. The areas under these curves are plotted as a calibration curve in Fig. 3.

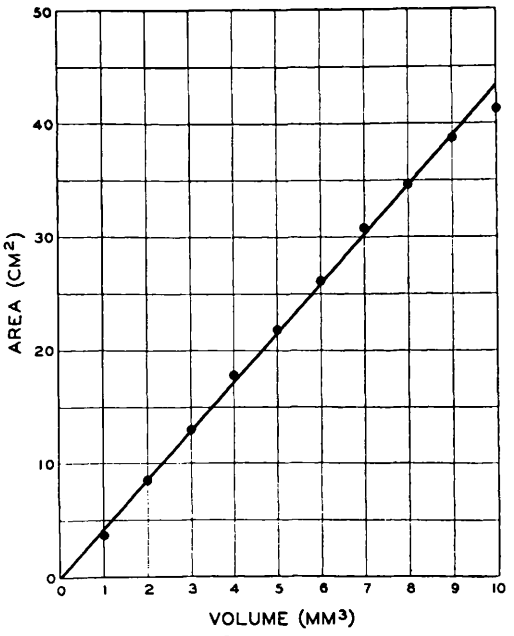

FIG. 3.
Calibration curve obtained from areas shown in Fig. 2.

mixed with normal rabbit serum appears to depend on a critical minimum of total protein placed on the chromatogram. Experiments in which labelled bovine serum albumin was mixed with varying quantities of unlabelled bovine serum albumin have shown the same dependence on a critical quantity of protein. A more complete treatment of these observations will be published in detail at a later date. The serum protein particularly rabbit serum albumin provided by 20 mm^3 of serum is well above the critical quantity necessary for the labelled albumin to move essentially as one spot.

In contrast are the patterns shown in Frames 3-8 which were obtained by superimposing 1 to 6 mm^3 of labelled albumin solution containing approximately 3.0 $\mu g/mm^3$ of albumin on 20 mm^3 of rabbit antiserum to bovine serum albumin. It will be seen that in Frames 3-6, representing 1 to 4 mm^3 of labelled antigen, the activity has remained fixed in a single peak at the starting point of the chromatogram. In contrast, when 5 mm^3 of albumin solution were added to 20 mm^3 of antiserum a significant amount of activity moved up the chromatogram. It appeared as (1) a long sloping take-off from the starting point peak (2) a low peak with an R_f value of 0.6 and (3) a small sharp peak moving with an R_f of 0.95 similar to bovine albumin moving on normal rabbit serum. At a level of 6 mm^3, Frame 8 shows a significantly larger total activity which has moved up the paper in peaks with R_f values of 0.28 and 0.95 plus a long valley of activity significantly above the base line. As a preliminary interpretation it seems reasonable to interpret the peaks with R_f values of 0.95 as free albumin or albumin complexed with such small ratios of antibody or other proteins as to have little retarding effect on its movement. The radioactivity peaks retained at the starting points

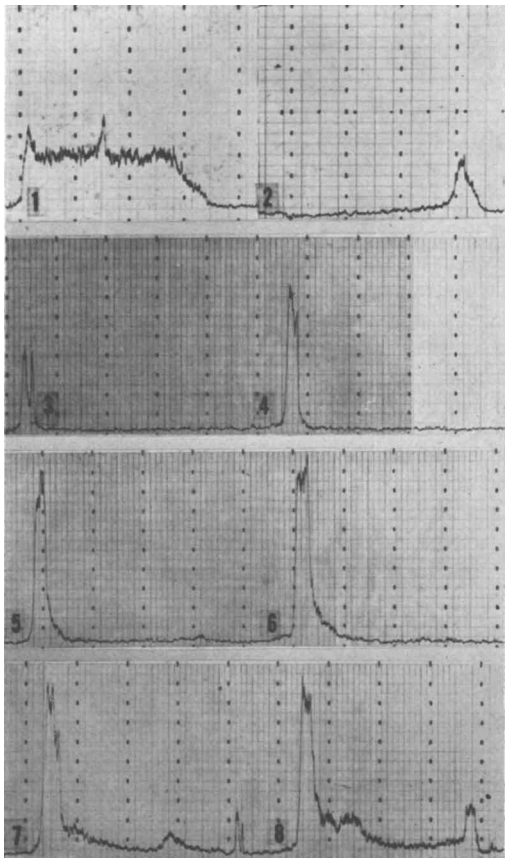

FIG. 4.
(1) Labelled bovine serum albumin in 0.15 M sodium chloride solution. (2) Labelled bovine serum albumin superimposed on normal rabbit serum. (3-8) 1-6 mm^3 of labelled bovine serum albumin solution superimposed on 20 mm^3 of rabbit antiserum to bovine serum albumin. Maximum area was found under curve 6.

of the chromatograms in frames 3-8 in which varying volumes of albumin solution were added to a constant volume of antiserum appear to represent labelled albumin immobilized on the chromatogram by virtue of its combination with antibody in an insoluble complex. Beyond the equivalence ratio of antigen to antibody, a fraction of the radioactive albumin remains combined in soluble antigen-antibody complexes. These move along the chromatogram, but at rates significantly different from labelled bovine serum albumin on normal rabbit serum.

As a check on the quantitative aspects of the chromatographic titration, the areas under the peaks at the starting points of the chromatograms were measured with a planimeter. There could be measured quantitatively because the spots left at the starting points of the chromatogram were very nearly circular. In order to evaluate the accuracy of the method, aliquots from the same I^{131} labelled albumin solution were used to provide a standard precipitin curve with the same antiserum used in the chromatographic titration. The precipitates were redissolved and made up to volume in 0.25 N sodium hydroxide solution for measurement of ultra-violet absorption at 290 mμ. Since in the chromatographic titration the quantity measured is labelled antigen retained on the starting spot and in the other the total antigen-antibody complex, the absolute values along the curve are not comparable. Therefore the ratios of antigen solution volume to antiserum volume at which maximum retention of antigen and maximum precipitation of antigen antibody complex occurred were compared. The volumes of each were corrected by the appropriate volume and dilution factors to the volume of antigen solution added per ml of antiserum and plotted on the same base line. These data plotted in Fig. 5 show maximum labelled albumin retention at the starting spot to occur at a ratio of 1.08 ml of albumin solution to 1.0 ml of antiserum as compared to 1.20 ml of the same albumin solution to 1.0 ml of antiserum for the precipitin titration.

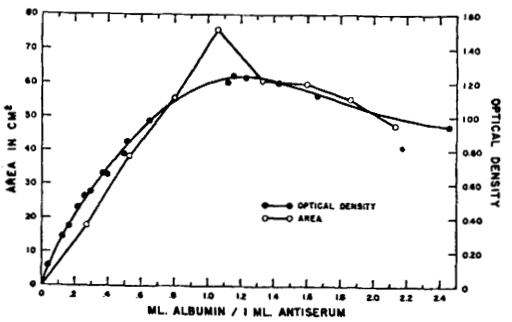

FIG. 5.
Simultaneously plotted are the optical densities at 290 mμ obtained from precipitates from 1 ml of rabbit anti-albumin antiserum and the areas from the starting point peaks from Fig. 4. Both are plotted against the same base line in terms of ml of the same albumin solution against 1 ml of the same antiserum.

Discussion. It has been shown by Eisen and Keston(6) and by Knox and Endicott(2) that lightly iodinated bovine serum albumin shows little deviation from native bovine serum albumin with respect to the precipitin curves obtained with antisera to native serum albumin. However in order to compare the methods as closely as possible aliquots of the same I^{131} labelled albumin solution were used for both titrations. It is encouraging to find that the antigen-antibody ratio at which maximum albumin activity is retained by the antiserum on the starting spot is only 11% lower than the ratio at which maximum precipitation is obtained by a standard method. It should be pointed out that the chromatographic titration provides a measure of the antigen precipitated rather than of the total antigen-antibody complex precipitated as in the usual method of determining a precipitin titre. Hence further check should be obtained using a labelled antiserum prepared in the manner described by Pressman and Sternberger(7). With a bland antigen and a radioactive antiserum a direct measurement of antibody would then be possible. The procedure requires much further study and refinement of method but appears promising not only as a quantitative tool for measurement of antigen antibody reactions, but also in the study of other protein-protein interactions. It may also furnish useful data in

6. Eisen, H. N. and Keston, A. S., *J. Immunol.*, 1949, v63, 71.

7. Pressman D. and Sternberger L. A., *J. Am. Chem. Soc.*, 1950, v72, 2226.

isolation procedures involving protein mixtures and complexes.

Summary. 1. A continuously recording counter adapted to the scanning of radio chromatograms has been described. 2. This method has been applied to a chromatographic method of demonstrating and measuring the reaction between radioactive iodine labelled bovine serum albumin and its antiserum. 3. Preliminary experiments have shown a reasonable agreement between the zone of maximum antigen fixation obtained by a chromatographic method and the zone of maximum precipitation obtained by the precipitin method. 4. The method appears to have useful possibilities in characterizing soluble antigen-antibody complexes as well as other protein-protein interactions. 5. It has been demonstrated that normal sera have the property of permitting a homogeneous movement of the labelled bovine serum albumin whereas the buffered solutions thus far tried have resulted in streaking of the albumin.

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Effect of 2,4-diamino-5-(p-chlorophenoxy)-6-methylpyrimidine and 2,4-diamino-6,7-diphenylpteridine on Chlorguanide-resistant Strain of *Plasmodium gallinaceum.* (18716)

JOSEPH GREENBERG AND EDNA M. RICHESON. (Introduced by R. W. Berliner)

From the Federal Security Agency, Public Health Service, National Institutes of Health, National Microbiological Institute, Bethesda, Md.

In previous reports(1,2), resemblances were shown to exist in the biological activities of certain biguanide-type compounds including chlorguanide (Paludrine), certain 2,4-diaminopyrimidines and 2,4-diaminopteridines. The antimalarial activity of these compounds against *Plasmodium gallinaceum* was potentiated by sulfadiazine and partially inhibited by folic acid. It was considered possible that the mode of action of these compounds might be identical, and one would therefore expect that a strain of parasites resistant to one of them would also be resistant to the others. Recently Falco *et al.*(3) have shown that some 2,4-diaminopyrimidines are over 700 times as active as quinine and 60 times as active as chlorguanide aginst *P. gallinaceum.* Such compounds are clearly of potential significance in the treatment of human malaria. Their application in this field might be limited, however, if strains of malaria now resistant to chlorguanide were also resistant to the pyrimidines.

This paper deals with the effect of one representative, 2,4-diamino-5-aryloxypyrimidine and one 2,4-diaminopteridine on a chlorguanide-resistant strain of *P. gallinaceum.*

Methods. Two strains of *P. gallinaceum* were used: a chlorguanide-resistant strain and its "normal" 8A parent strain. According to the criteria used in this laboratory(4) the chlorguanide-resistant strain was, at the time of these experiments, about 16 times more resistant to the activity of the drug than was the parent strain. The hosts were week-old New Hampshire Red chicks weighing 45 to 55 g. The chicks were inoculated at random; each received 16 x 10^6 parasitized erythrocytes intravenously in 0.1 cc of inoculum. Drugs were administered twice daily for 4 days beginning 4 to 5 hours before inoculation. Blood smears were made and stained with Giemsa the morning following the last dose of drug, and parasite counts were made on the basis of parasitized cells per 10^4 erythrocytes. The

1. Greenberg, J., *J. Pharm. and Exp. Therap.,* 1949, v97, 484.

2. Greenberg, J., *J. Pharm. and Exp. Therap.,* 1950, v99, 444.

3. Falco, E. A., Goodwin, L. C., Hitchings, G. H., Rollo, I. M., and Russell, P. F., *Brit. J. Path.,* in press.

4. Coatney, G. R. and Sebrell, W. H., in Wiselogle's *A Survey of Antimalarial Drugs,* 1941-1945. 1946, J. W. Edwards, Ann Arbor, Mich.